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ANTI-DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF *WATTAKAKA VOLUBILIS* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Wattakaka volubilis(Linn. F.) Benth ex. Hook f. (Asclepidaceae) is a well-known medicinal plant and is used in various human ailments. In the present study investigated methanolic extract of *W. volubilis* for 21 days on the functions of hyperglycemia, hyperinsulinemia, hyperlipidermic and serum hepatic marker enzymes were evaluated with STZ induced diabetic mellitus in male wistar albino rats. They showed results that a significant increase in the levels of blood glucose, urea, creatinine and glycosylated Hb and decrease in the level of plasma insulin in the diabetic control rats were observed. A reduce in the activities of serum protein, albumin and globulin in diabetic rats and these levels were increased in *W. volubilis* treated groups III &IV. The marker enzymes SGPT, SGOT and ALP levels were significantly elevated in the STZ treated groups and compared to normal. The groups treated with *W. volubilis* methanolic extracts showed significantly decrease in the serum marker enzymes. The antidiabetic effect of *W. volubilis* extract was compared with a standard antidiabetic drug glibenclamide. It is concluded that methanolic extracts of *W. volubilis* possesses significant antidiabetic, antihyperlipidaemic and antioxidant effects in STZ induced diabetic rats.

INTRODUCTION

Diabetes mellitus is now considered to be a worldwide epidemic and without primary prevention, the epidemic will continue unabated. Herbal drugs constitute an important part of traditional medicine and literature shows that there are more than 400 plant species showing

antidiabetic activity (Arulrayan*et al.*, 2007). Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost. The most potent hypoglyceamic effects are commonly found in *Trigonella foenumgraecum* L. (Fabaceae), *Cheilocostus speciosus* (Costaceae), *Camellia sinensis*(Theaceae), *Momordica charantia* Linn. (Cucurbitaceae), *Gymnema sylvestre* (Apocynaceae), *Aegle marmelose* L. (Rutaceae), *Tinospora crispa* (Menispermaceae), *Azadirachta indica* L. (Meliaceae) *Polygala senega* L. (Polygalaceae) (Bnouham *et al*.,2006).

W. volubilis belongs to the family Asclepiadaceae. The plants are distributed along subtropical of Malaysia, India, South China, Taiwan and Srilanka. *W. volubilis* leaves were examined for their antidiabetic efficacy. *W. volubilis* widely practiced in Indian traditional medicines and the leaf paste to treat rheumatic pain, cough, fever and wicked cold (Muthu *et al.*,2006). Bark paste, mixed with hot milk is utilized internally for treating urinary troubles and leaf powder is taken orally along with cow's milk have antidiabetic activity (Silija *et al.*,2008; Ayyanar *et al.*,2008).

MATERIALS AND METHODS

Collection of plant material

The fresh plant *W. volubilis* collected from Velliangiri hills, Coimbatore, Tamil Nadu. The plants were identified and authenticated by a plant taxonomist.

Preparation of extract

250 g of collected plant sample were washed with 2-3 times water followed by distilled water and shade dried. The dried parts of plants were pulverized by mechanical grinder (Willy mill) to get the powder through 100 mesh sieve and then stored in a desiccator. The shade dried and powdered plant materials were extracted with petroleum ether to remove the resins and the residue was then extracted with methanol by using soxhlet apparatus.

Experimental Induction of Diabetes

Male albino rats weighing about (120-200gm) were used in this present study. They were kept in a 12:12hr Light: Dark cycle and the temperature maintained at 22°±2°C. Standard laboratory pellet diet (Hindustan Lever, Bangalore) and water were given *adlibitum*. The care of the animals was as per the 'Guidelines for the care and use of animals in the scientific research'by the Indian National Science Academy New Delhi (Ravikumar and Santhosh,2008). STZ injected animal exhibited massive glycosuria and hyperglycemia within

a few days. Diabetes was confirmed in the overnight –fasted rats by measuring blood glucose concentration. The rats with blood glucose above 250 mg dL^{-1} were considered to be diabetic and used further experiment.

Experimental Design

30 adult male albino rats weighing about (120-200g) were divided into five groups, six rats each. Group I: Rats given normal saline daily for 21 days consequently orally (by using an intragastric catheter tube (IGC). (Normal control), Group II: Diabetic rats given normal saline daily for 21 days consequently orally by using IGC (Diabetic control), Group III: Diabetic rats given *W. volubilis* methanolic extract at the dose of 150µg/kg body weight daily for 21 days consequently, orally for 21 days by using IGC (Drug treated), Group IV: Diabetic rats given *W. volubilis* methanolic extract at the dose of 300µg/kg body weight daily for21 days consequently, orally for 21 days by using IGC (Drug treated), Group V:Diabetic rats given glibenclamide at the dose of 400µg kg^{-1} body weight daily for 21 days consequently, orally for 21 days by using IGC (Drug treated).The dose (150 & 300 mg kg^{-1}B.Wt.), orally by IGC was standardized by pilot study with different doses of methanolic extract of *W. volubilis* to assess the antihyperglycemic effects in STZ induced diabetic rats. After the experimental period all the rats were sacrificed by cervical dislocation and biochemical studies conducted in Blood, Plasma and liver samples. The plasma and liver samples was assayed by ELISA method using boehringer mannheim gmbh kit. The data were expressed as mean ± SD. Statistical comparisons were performed by one-way Anova followed by Dunnelt's test. The results were considered statically significant if $P<0.05$.

RESULTS

Antidiabetic activity

Glucose, Urea, Insulin and Creatinine

All the diabetic rats groups II, III and IV had significantly higher ($p<0.001$) levels of plasma glucose, urea and creatinine than in control groups, indicating a poor control of diabetes. There was a significant reduction in serum insulin levels in STZ diabetic rats, compared with normal rats. Administration of *W.volubilis* methanolic extract at 300µgkg^{-1} body wt., and glibenclamide tended to bring blood glucose and serum insulin towards normal levels. Treatment of rats with *W. volubilis* methanolic extract produced significant ($p<0.05$) decrease in the urea and serum creatinine levels in comparison with the control group. The diabetic rats administrated with *W. volubilis* methanolic extract at 300 µg kg^{-1} body wt., and

glibenclamide at the dose of 400 µg kg^{-1} body wt., daily for 21 days consequently orally by IGC altered the values of insulin, glucose and when compare to control. All the results were compared with the standard drug, glibenclamide (Table-1).

Glycosylated Heamoglobin

The level of glycosylated haemoglobin profile of different experimental groups of untreated and treated animals are represented in Table-1. The diabetic rats showed a significant increase in the level of glycosylated haemoglobin. The administration of *W. volubilis* methanolic extract at the dose of 300 µg kg^{-1}b.wt., given to the diabetic rats restored the changes in the level of glycosylated haemoglobin to near normal level ($P<0.01$).

Blood Glucose levels

The effect of extracts on the blood glucose level measured in I, II, III and IV week until the levels returned to those measured prior to the treatment. The table- 2 shows a prolonged effect in animals which received the *W. volubilis* methanolic extract at the dose of 300 µg kg^{-1} body wt., blood glucose levels were 167.54 ± 0.49 on I week, 133.62±3.24 on II week, 109.2±3.01 on III week and 86.25±2.94on IV week. The *W. volubilis* methanolic extract treated group has shown good hypoglycaemic activity compared to the standard drug treated group.

Protein, Albumin and Globulin

The table-3 shows the effect of *W. volubilis* methanolic extract administration on STZ induced diabetes for 21 days. The STZ diabetic induced groups showed a significant decrease in protein, albumin and globulin levels were compared with the controls. The administration of *W. volubilis*methanolic extract at the dose of 300 µg kg^{-1}body wt., showed a significant ($p<0.05$) elevation in the levels of protein, albumin and globulin levels when compared to Group II. The administration of the *W. volubilis* methanolic extract and glibenclamide tended to bring the values to rear normal. The diabetic rats showed a significant elevation in the total bilirubin, when compared to the control group. However the *W. volubilis* methanolic extract treated groups showed a significant decrease in the levels of total bilirubin. The *W. Volubilis* methanolic extract treated groups were showed momentous activity comparable to the standard drug treated group.

SGOT, SGPT and ALP

The STZ induced diabetic groups showed a significant hepatic damage as observed from elevated serum levels of hepatospecific enzymes as well as severe alterations in different liver parameters. Activities of SGOT, SGPT and alkaline phosphatase in serum were increased in the diabetic control group animals. Where the *W. volubilis* methanolic extract treated groups observed the protection of liver which reduces the leakage of enzymes SGOT, SGPT and alkaline phosphatase into serum. The effect of *W. volubilis* methanolic extract at the dose of 300 µg kg^{-1}body wt., treated group was prominent when compared with glibenclamide (Table-3).

Lipid profile

The lipid profile such as TC, TG, LDL, PL and VLDL levels were significantly increased in diabetic control animals (DC) where as HDL levels were decreased when compared to the control rats (Table-4). The *W. volubilis* methanolic extract was administered orally at dose level of 300 µg kg^{-1}b.wt., to diabetic rats. The diabetic animals at 300 µg kg^{-1}dosage recorded a significant ($P<0.001$) depletion in the total cholesterol level was recorded in the diabetic animals. The depletion in the TC, TG, LDL, PL and VLDL were dose dependent and the highest reduction in the cholesterol was recorded (130.31±1.90), TG (91.50±2.28), LDL (64.88±2.43), PL (181.17±3.96) and VLDL (19.41±1.18) in the *W. volubilis* methanolic extract treated, when compared to the diabetic control animals. The depleted high density lipoprotein (HDL) in the diabetic rats, increased significantly ($P<0.001$) after the administration of the plant extracts. The highest increase was recorded in *W. volubilis* methanolic extract at 300 µg kg^{-1}body wt., dosage level (50.31 ± 1.63).

SOD, CAT, GPx, GSH and LPO

The enzymatic and non-enzymatic antioxidant levels of serum, liver tissues and kidney tissues were represented in table-5. A significant decrease in the activities of antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and GSH and increase in the levels of lipid peroxides with a concomitant decrease in the levels of total reduced glutathione in the Group II rats indicate the severity of oxidative stress induced as result of administration of STZ. Considerable increase in the activities of antioxidant enzymes, decrease in the levels of lipid peroxides and improvement in hepatic GSH status in the *W. volubilis* methanolic extract at the dose of 300 µg kg^{-1}b.wt.

Table 1: Effect of *W. volubilis* methanolic extracts on the Insulin, blood glucose, urea and creatinine levels of normal, diabetic induced and drugs treated rats

Parameter	Insulin (MIu/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	HbA_1C (%)
Group I	18.36±0.8	87.63±2.42	14.91±1.76	0.81±0.16	4.36 ± 0.08
Group II	7.86±0.31*	214.13±8.54**	35.65 ± 3.11*	3.54±0.17**	17.34±1.23**
Group III	14.84±0.36a	131.28±7.24*a	26.28 ± 1.62	1.73±0.73	8.11±0.20
Group IV	15.12±1.46a	118.27±3.28a	30.40 ± 2.11	1.21±0.53	5.54±0.27aa
Group V	19.74±2.16aa	76.83±1.84aa	19.13±2.65a	1.43±1.17a	7.63 ± 0.17aa

Each Value is * SEM of 6 animals

*P<0.05, ** P<0.01- Comparison between Normal control Vs other treated groups; a P<0.05; aa P<0.01 - Comparison between diabetic control Vs other treated groups

Table 2: Effect of *W. volubilis* methanolic extracts on blood glucose level of normal, diabetic induced and drug treated rats at different time intervals.

Groups	Blood glucose level in mgs/dl				
	0 day	**1 week**	**2 week**	**3week**	**4 week**
Group I	79.63±0.73	86.21 ± 4.86	96.21±1.89	82.33±2..01	81.50 ± 1.61
Group II	211.39±5.01**	233.81±5.96**	236.47±3.41**	211.22±5.22**	227.15±5.47**
Group III	225.34 ± 6.37**	225.34 ± 6.31**	185.53 ± 0.23**	157.52±4.97*a	97.37±4.63aa
Group IV	217.22±6.73**	167.54 ± 0.49*a	133.62±3.24*aa	109.2±3.01aa	86.25±2.94aa
Group V	181.1±3.37**	139.11 ± 0.48*	117.27±4.27aa	95.31 ± 1.27aa	92.69±1.99aa

Each Value is * SEM of 6 animals * P < 0.05

*P<0.05, ** P<0.01- Comparison between Normal control Vs other treated groups; a P<0.05; aa P<0.01 - Comparison between diabetic control Vs other treated groups

Table 3: Effect of *W. volubilis* methanolic extracts on the protein, albumin, globulin, SGOT, SGPT and ALP levels of normal, diabetic induced and drug treated rats.

Parameter	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	6.95 ± 0.51	4.16±0.13	3.14±0.23	27.81±3.44	24.52±1.89	153.36 ± 4.81
Group II	6.02±0.13*	2.73±0.13*	2.39±0.17*	172.0±3.51**	197.91±3.81*	-
Group III	6.34±0.36ns	3.59±0.69	3.43±0.26	88.96±3.21a	103.05±2.07a	105.83±2.01a
Group IV	8.11±0.90	4.90±0.14	3.02±0.18	40.01±3.90aa	50.50±2.10aa	113.19±3.37a
Group V	9.84±0.27a	4.70±0.20	3.80±0.19	37.48±3.30aa	33.33±2.68aa	99.67±2.19aa

*P<0.05, ** P<0.01- Comparison between Normal control Vs other treated groups; a P<0.05; aa P<0.01 - Comparison between diabetic control Vs other treated groups

Table 4: Effect of *W. volubilis* methanolic extracts on the TC, TG, LDL-C, HDL, VLDL and PL in the plasma of normal, diabetic induced, and drug treated rats.

Paramete	TC (mg/dl)	TG(mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	PL (mg/dl)
Group I	117.83±3.61	74.36 ± 1.7	46.21±2.14	14.04±0.68	54.39 ± 2.92	170.20±4.94
Group II	173.90±4.31**	129.71±5.13**	131.48±4.56**	27.14±1.23*	26.51±2.49**	-
Group III	137.3±3.17a	83.20±4.90aa	63.99±2.07a	16.10±1.41	44.50±1.93	183.99±3.98*
Group IV	130.31±1.90aa	91.50±2.28aa	64.88±2.43a	19.41±1.18	50.31 ± 1.63a	181.17±3.96
Group V	116.27±2.17aa	78.79±1.49aa	50.48±2.73aa	22.46±1.16	51.50±1.80aa	176.74±6.31

*P<0.05, ** P<0.01- Comparison between Normal control Vs other treated groups; a P<0.05; aa P<0.01 - Comparison between diabetic control Vs other treated groups

Table 5: Effect of *W. volubilis* methanolic extracts on serum LPO, GPX, GSH, SOD and CAT in the normal, diabetic and drug treated rats.

Groups	Parameters				
	LPO (nanomol/mg protein)	**GPX (u/mg protein)**	**GSH (u/mg protein)**	**SOD (u/mg protein)**	**CAT (u/mg protein)**
Group I	3.01±0.014	521.32±38.97	31.34±1.87	473.18±15.37	87.33±1.73
Group II	5.17±0.052**	420±18.07**	20.32±1.97**	271.39±17.32**	46.21±2.14**
Group III	3.74±0.014ns	412±15.91*a	27.20±1.18	275.30±16.20	60.47±1.70
Group IV	3.68±0.071aa	527±15.37aa	36.57±2.51a	412.23±14.25a	71.70±2.20a
Group V	2.17±0.033aa	557±17.61a	45.10±2.78aa	654.78±30.03aa	81.75±1.98aa

*P<0.05, ** P<0.01- Comparison between Normal control Vs other treated groups; a P<0.05; aa P<0.01 - Comparison between diabetic control Vs other treated groups

DISCUSSION

Diabetes may be induced by excessive consumption of alcohol or fatty, sweet, pungent or fried foods. The excess fat transforms into interior dampness-phlegm, accumulates and impairs yin fluid thereby prevents food essence from nourishing the muscles, skin, lungs, liver, kidney and stomach (Chen, 1992). Several plant phytoconstituents are known to reduce TG (Lee *et al.*, 2000) which is usually increased in the serum of diabetes (Arkkila*et al.*, 2001). Such a significant increase in TG may be due to the lack of insulin under diabetic condition, while insulin activates the enzyme lipoprotein lipase and hydrolysis TG under normal condition.

Diabetes affects both glucose and lipid metabolism (Sperling *et al*., 2000). In the post prandial state elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism (Bhagavan, 2002). The insulin

deficiency depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes (Ranganathan *et al.*, 2000). The lipoprotein levels in the STZ induced diabetic rats of the present study reveal a significant alter in lipoprotein metabolism. The serum total cholesterol content (TC) increased significantly in diabetic animals. The elevated hypertriglyceridemia was increased in the synthesis of triglyceride rich lipoprotein particles (very low density lipoprotein, VLDL) in liver diminished catabolism in diabetic rats (Ginsberg, 1991). Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver (Coppack *et al.*, 1994, Ohno *et al.*, 2000) The increased levels of low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) in the diabetic animals might be due to over production of LDL and VLDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx (Coppack *et al.*, 1994). The high density lipoprotein (HDL) was significantly reduced in the diabetic rats which indicate a positive risk factor for atherosclerosis (Bopanna *et al.*, 1997).Numerous studies have reported on a marked decrease in the activity of antioxidant enzymes such as GPx and CAT as well as GST activity in liver, kidney and pancreatic tissues from diabetic rats (Latha and pari, 2003). Few reports stated that the flavonoids, sterols ,terpenoids, phenolic acids are recognized to be bioactivities antidiabetic principles (Silija *et al*.,2008). Flavonoids are known to restore the damaged beta cells in the alloxan diabetic rats (Bergmayer,1983). Phenolics are found to be effective antihyperglycemic agents (Pagila and Valentine,1967).

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