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ANTIDIABETIC, ANTIHYPERLIPIDAEMIC AND ANTIOXIDANT ACTIVITY OF *WATTAKAKA VOLUBILIS* (L. F) STAPF LEAVES IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The ethanol extract of *Wattakaka volubilis* (L. f) Stapf. (Family: Asclepiadaceae) leaf was investigated for its antidiabetic effect in Wistar Albino rats. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg, i.p). The ethanol extract of *Wattakaka volubilis* at a dose of 150mg/kg of body weight was administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of *Wattakaka volubilis* leaf extract on blood glucose, plasma insulin, glycosylated haemoglobin, serum lipid profile [total cholesterol, triglycerides, low density lipoprotein - cholesterol (LDL-C), very low density lipoprotein - cholesterol (VLDL-C), and high density lipoprotein- cholesterol (HDL-C) serum protein, albumin, globulin, A/G ratio, serum enzymes [Serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transaminases (SGOT) and alkaline phosphatase (ALP)], lipoprotein peroxidation (LPO) antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) ,glutathione peroxidase (GPx) and glutathione reductase (GR) were measured in the diabetic rats. The ethanol extract of *Wattakaka volubilis* leaf elicited significant reductions of blood glucose ($p<0.01$), lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C and antioxidant enzymes. The extracts also caused significant increase in plasma insulin ($p<0.01$) in the diabetic rats. From the above results it is concluded that ethanol extract of *Wattakaka volubilis* possesses significant antidiabetic, antihyperlipidaemic and antioxidant effects in alloxan induced diabetic rats.

INTRODUCTION: Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alterations in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and/or insulin action [1]. It is one of the oldest diseases affecting millions of people all over the world [2]. According to recent estimates the prevalence of diabetes mellitus is 4% worldwide and that indicates 143 million persons are affected which will increase to 300 million by the year 2025 [3]. Although numerous oral hypoglycemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes [4]. Over the last few decades the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety. In recent years, numerous traditional medicinal plants were tested for their antidiabetic potential in the experimental animals [5].

In the present investigation, *Wattakaka volubilis* (L. f) Stapf. leaves were tested for their antidiabetic efficacy. *Wattakaka volubilis* (L.f) Stapf (Family: Asclepiadaceae) is widely used in Indian traditional medicines and the leaf paste to treat rheumatic pain, cough, fever and severe cold [6, 7]; leaf paste is taken along with pepper to treat dyspepsia [8]; bark paste, mixed with hot milk is used internally for treating urinary troubles [9] and leaf powder is taken orally along with cow's milk have antidiabetic activity [10]. In view of above medicinal properties, the present study was designed to investigate the antidiabetic efficacy of ethanolic extract of *Wattakaka volubilis* leaf in alloxan induced diabetic rats.

MATERIALS AND METHODS:

Plant material: *Wattakaka volubilis* (L.f) Stapf leaves were freshly collected from the Sirumalai hills, Western Ghats, Tamil Nadu. The plant were identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for Phytochemical Screening and Antidiabetic Studies: The *Watta-kaka volubilis* leaves were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *Wattakaka volubilis* leaves was packed in a Soxhlet apparatus and extracted with ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures [11, 12, 13]. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

Animals: Normal healthy male Wistar albino rats (180-240g) were used for present investigation. Animals were housed under standard environmental conditions at temperature (25±2°C) and light and dark (12:12 h). Rats were feed standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Study: Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [14]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated

again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100, and 1000 mg/kg body weight.

Induction of Experimental Diabetes: Rats were induced diabetes by the administration of simple intraperitioneal dose of alloxan monohydrate (150 mg/kg) [15]. Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycaemia with blood glucose level of 200-260 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental Design: In the investigation, a total of 24 rats (18 diabetic surviving rats and 6 normal rats) were taken and divided into four groups of 6 rats each.

Group I: Normal, untreated rats

Group II: Diabetic control rats

Group III: Diabetic rats given ethanol extract of *Wattakaka volubilis* leaf (150 mg/kg of body weight)

Group IV: Diabetic rats given standard drug glibenclamide (600µg/kg of body weight)

Biochemical Analysis: The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes. Serum glucose was measured by the O-toluidine method [16]. Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit [17]. Glycosylated haemoglobin (HbA_1C) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan [18]. Serum total cholesterol (TC) [19], total triglycerides (TG) [20], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) [21] and high density lipoprotein cholesterol (HDL-C) [22] were analyzed. Serum protein [23] and serum albumins was determined by quantitative colorimetrically method by using bromocresol green.

The total protein minus the albumin gives the globulin, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel [24]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong [25]. Catalase (CAT) [26], superoxide dismutase (SOD) [27], lipid peroxidation (LPO) [28], reduced glutathione (GSH) [29], glutathione peroxidase (GPx) [30] and glutathione reductase (GR) [31] were analyzed in the normal, diabetic induced and drug treated rats.

Statistical Analysis: The data were analyzed using student's t-test statistical methods. For the statistical tests a p values of less than 0.01 and 0.05 was taken as significant.

RESULTS AND DISCUSSION: The phytochemical screening of ethanol extract of *W. volubilis* leaf revealed the presence of alkaloids coumarins, flavonoids, glycosides, terpenoids, tannins, phenols, saponins and steroids. Acute toxicity study revealed the non-toxic nature of the ethanol extract of *W. volubilis* leaf. The alloxan induced diabetic rats elicited significant rise in blood glucose from 75.31 to 225.34 mg/dl ($p<0.01$) and a significant decrease in plasma insulin level from 19.51 to 4.31 ($p<0.01$).On the contrary, diabetic rats treated with ethanol extract of *W. volubilis* exhibited decrease blood glucose and increase the plasma insulin significantly at a dose of 150 mg/kg body weight (**Table 1**).

TABLE 1: EFFECT OF ETHANOL EXTRACT OF *WATTAKAKA VOLUBILIS* LEAF ON SERUM GLUCOSE, INSULIN AND GLYCOSYLATED HAEMOGLOBIN OF NORMAL, DIABETIC INDUCED AND DRUG TREATED RATS

Parameter	Glucose (mg/dl)	Insulin (µg/dl)	HbA_1C (%)
Group I	75.31±5.1	19.51±0.66	3.90±0.1
Group II	225.34±6.3**	4.31±0.36**	11.4±1.2**
Group III	128.14±3.8	10.31±2.3	8.46±5.4*
Group IV	102..21±1.4[a]	14.32±2.4	7.31±1.2

Each value is SEM of 6 animals, Comparisons were made between normal control to diabetic control and drug treated:*p < 0.05; **p<0.01 and comparisons were made between diabetic control to drug treated groups: [a] p<0.05 level

The hypoglycemic ethanol effect of *W. volubilis* leaf was found to be inducing insulin release from pancreatic cells of diabetic rats[32]. Earlier many plants have been studied for their hypoglycemic and insulin release stimulatory effects[33-35]. Alloxan induced diabetic rats showed significant increased (p<0.01) glycosylated haemoglobin (HbA_1C) level compared with normal rats. The ethanol extract of *W. volubilis* leaf treated rats showed a significant decrease (p<0.05) in the content of glycosylated haemoglobin. Glycosylated haemoglobin determinations are self monitoring of blood glucose therefore play important complementary roles for the management of diabetes mellitus [36]. The levels of serum protein, albumin and globulin of control and alloxan induced diabetic rats were presented in **Table 2**.

TABLE 2: EFFECT OF ETHANOL EXTRACT OF *WATTAKAKA VOLUBILIS* LEAF ON THE SERUM PROTEIN, ALBUMIN, GLOBULIN, SGOT, SGPT AND ALP LEVEL OF NORMAL, DIABETIC INDUCED AND DRUG TREATED RATS

Parameter	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	7.31 ± 0.28	4.11± 0.86	3.26 ± 0.84	1.26:1	13.26 ± 1.14	17.43 ±1.99	153.36 ± 4.89
Group II	5.23 ± 0.31*	3.16 ± 0.19	2.07 ±0.34	1.53:1	32.89±7.34*	29.22±1.32	298.45±6.98*
Group III	6.25 ± 0.33	4.14 ± 0.21	2.91 ±0.12	2.1:1	30.45 ±3.92*	20.34±1.54	231.98±7.08*
Group IV	7.63 ± 0.11[a]	3.96 ± 0.62	3.67 ± 0.45	1.1:1	16.34 ±1.78	14.78±1.04	164.22±6.39[a]

Each value is SEM of 6 animals, Comparisons were made between normal control to diabetic control: * p < 0.05 and comparisons were made between diabetic control to drug treated groups: [a] p<0.05 level

A significant reduction in serum protein, albumin and globulin were observed in alloxan induced diabetic rats (Group II) when compared to control (Group I) and glibenclamide treated rats (Group IV). On administration of ethanol extract of *W. volubilis* leaf to the diabetic rats (Group III), the levels of protein, albumin and globulin were found to be restored in normal. These results were in accordance with the effect of *Artemisia herba-alb*a and *Teucrium polium* in diabetic rats [37]. **Table 2** summarized the effect of alloxan on the activity of the hepatic marker enzymes in serum. In the present study, the levels of SGPT and SGOT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of . In this study, the ethanol extract of *W.volubilis* leaf regulated the activity of SGPT and SGOT in liver of rats intoxicated with alloxan. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study [39]. The restorations of SGPT and SGOT to their respective normal levels after treatment with both glibenclamide and ethanol extract of *W.volubilis* further strengthen the

antidiabetogenic effect of this extract. Moreover SGPT and SGOT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver. Since the alloxan can also affect the liver by free radical mechanism.

In addition to the assessment of SGPT and SGOT levels during diabetes the measurement of enzymatic activities of phosphatases such as acid phosphatase (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants [40]. In the present study, serum ALP increased considerably ($p<0.05$) in alloxan induced diabetic rats. Elevated level of this enzyme in diabetes may be due to extensive damage to liver in the experimental animals by alloxan.

Treatment with ethanol extract of *W. volubilis* in alloxan induced diabetic rats produces a significant ($p<0.05$) decline in ALP level. The levels of serum lipid profiles, total cholesterol (TC), triglycerides (TG), LDL-C, VLDL-C, and HDL-C in control and experimental animals were investigated (Table 3). Alloxan induced rats showed significantly increased serum lipid profiles except HDL-C when compared with normal rats. The glibenclamide and ethanol extract of *W. volubilis* leaf treated rats showed a significant decrease in the content of lipid profiles when compared with diabetic induced rats. Similarly HDL-C level decreased in alloxan induced diabetic rats when compared to normal rats. On administration of ethanol extract of *W. volubilis* leaf and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normal. The level of serum lipid profiles are usually raised in diabetic rats in the present study and such elevation represents risk factor for coronary heart diseases [41]. The hypolipidemic effect may be due to inhibition of fatty acid synthesis [42]. In normal metabolism insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia. The significant reduction of serum lipid levels in diabetic rats after *W. volubilis* treatment may be directly attributed to improvements in insulin levels.

The results (**Table 4**) showed increased lipid peroxidation (LPO) of alloxan induced diabetic rats. Earlier studies have reported that there was an increased lipid peroxidation in liver, kidney and brain of diabetic rats [43, 44]. In the present study, an increase in the levels of LPO ($p<0.05$) was found and these levels were significantly reduced after the supplementation of the ethanol extract of *W. volubilis* leaf and glibenclamide. These indicate that, plant extract inhibit oxidative damage due to the antiperoxidative effect of ingrediants present in ethanol extract of *W. volubilis* leaf. This could be correlated with previous study with *Cassia auriculata* flower [45] and *Scoparia dulcis* [46].

TABLE 3: EFFECT OF ETHANOL EXTRACT OF *WATTAKAKA VOLUBILIS* LEAF ON SERUM LIPID PROFILE OF NORMAL, DIABETIC INDUCED AND DRUG TREATED RATS

Parameter	TC (mg/dl)	TG (mg/dl)	LDL – C (mg/dl)	VLDL – C (mg/dl)	HDL – C (mg/dl)
Group I	92.16 ± 1.6	74.36 ± 1.7	25.08 ± 0.98	14.87 ± 1.31	53.21 ± 1.93
Group II	156.23 ± 2.6**	189.41 ± 5.3*	86.21 ± 4.86*	37.88 ± 2.45*	32.14 ± 2.61*
Group III	123.52 ± 2.1	133.4± 2.4	53.41 ± 2.49	22.68 ± 1.04	47.43 ± 2.36
Group IV	96.26± 1.9[a]	81.50 ± 1.6[a]	30.40 ± 2.11	16.32 ± 1.64	49.54 ± 1.92

Each value is SEM of 6 animals, comparisons were made between normal control to diabetic control: *$p<0.05$; **$p<0.01$ and comparisons were made between diabetic control to drug treated groups: [a] $p<0.05$ level

TABLE 4: EFFECT OF ETHANOL EXTRACT OF *WATTAKAKA VOLUBILIS* LEAF ON THE CAT, SOD, LPO, GSH, GPX, AND GR ACTIVITY OF NORMAL, DIABETIC INDUCED AND DRUG TREATED RATS

Parameter	Erythrocytes		Blood serum			
	CAT (mM/mgHb)	SOD (U/g Hb)	LPO (nmol/ml)	GSH (mM/ml)	GPx (µmol/ml)	GR (nmol/ml)
Group I	92.31±1.36	514.21±42.21	1.22±0.21	32.44±2.41	754.97±32.13	15.89±0.78
Group II	32.15±1.89*	212.26±39.87*	2.01±0.34*	21.32±1.98*	256.54±29.09*	10.56±0.87*
Group III	39.26±1.06	301.34±41.43[a]	1.99±0.31	20.44±1.68	302.31±29.87	11.23±0.65
Group IV	77.17±1.23[a]	489.21±36.76[aa]	1.21±0.12	30.45±2.33	654.78±30.03[a]	13.98±0.34

Each value is SEM of 6 animals, comparisons were made between normal control to diabetic control: *p<0.05 and comparisons were made between diabetic control to drug treated groups: [a] p<0.05; [aa] p<0.01 level

The levels of superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx) reduced glutathione (GSH) and glutathione reductase (GR) were significantly (p<0.05) reduced in alloxan induced rats. These adverse changes were reversed to near normal values in ethanol extract of *W. volubilis* leaf treated. It is well known that CAT, SOD and GPx play an important role as protective enzymes against free radical formation in tissues [47]. The present study indicates the reduction in the activity of SOD, CAT, GPx, GSH and GR in alloxan induced rats. These results reveal the protective role of plant extract in decreasing lipid peroxidation and by normalizing antioxidant system.

In conclusion, the present study has shown that the ethanol extract of the leaves of *W. volubilis* has antidiabetic and antihyperlipidaemic effects. Since the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, terpenoids, tannins, glycosides, sterols, phenols and saponins. Several authors reported that flavonoids, sterols/terpenoids, phenolic acids are known to be bioactive antidiabetic principles [48, 49]. Flavonoids are known to regenerate the damaged beta cells in the alloxan diabetic rats [50]. Phenolics are found to be effective antihyperglycemic agents [51]. In the present study, the phytochemical analysis of ethanol extract of *W. volubilis* leaf clearly points out the presence of above said active phytochemicals. It denotes that, the antidiabetic effect of ethanol extract of *W. volubilis* leaf may be due to the presence of more than one antihyperglycemic principle and their synergistic effects.

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