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International Journal of Pharma Research and Health Sciences

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Original Article

Antidiabetic and Antioxidant Properties of *Waltheria indica* L., an Ethnomedicinal Plant

M Kannan[1], T Senthil Kumar [2,*], M V Rao[3]

[1]Department of Botany, Directorate of Distance Education, Vinayaka Missions University,Salem-636 308, Tamil Nadu, India.
[2]Department of Industry University Collaboration, Bharathidasan University, Tiruchirappalli -620 024, Tamil Nadu, India.
[3]Department of Plant Science, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India.

ARTICLE INFO

Received: 07 Oct 2016
Accepted: 22 Oct 2016

ABSTRACT

The present investigation was carried out to study the antidiabetic activity of methanolic extract of entire plant of *Waltheria indica* L. and its effect on the lipid profile, antioxidant properties and histopathology of liver and kidney tissues of alloxan induced diabetic models. Studies were carried out in normal and alloxan induced diabetic rats treated with doses 200mg/kg and 400mg/kg of *Waltheria indica* L. extract where, Carboxy Methyl Cellulose (CMC) was acted as vehicle medium and Glibenclamide was used as standard drug. Decreased blood glucose level of the test animals showed that the extract has significant antidiabetic activity over the diabetic control group. The results also indicated dose dependent effect. The antidiabetic properties of the extract may be due to increased uptake of glucose at the tissue level. The extract also produced a significant increase in High Density Lipoprotein (HDL), Super Oxide Dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH) and Glutathione Peroxidase (GPx); significant decrease in Total Cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), Lipid Peroxidation (LPO), in test groups. This clearly represents the effect of test drugs in the antioxidant property and lipid profile of the test animals. The results obtained from the experiment provided the scientific support to the enthnomedicinal use of *Waltheria indica* L. for the treatment of diabetes mellitus.

Keywords: Alloxan, Antidiabetic activity, Antioxidant properties, Ethnomedicine, Glibenclamide, Histopathology, Kalrayan hills, Lipid profile, *Waltheria indica.*

Corresponding author *
T Senthil Kumar
Bharathidasan University, Tiruchirappalli -620 024, Tamil Nadu, India
Email: senthil2551964@yahoo.co.in

1. INTRODUCTION

Since, time immemorial man has used various parts of plants in the treatment of many diseases. Each culture or community with in an area, whether large or small, has its own ethnobotanical perspective, which differs from one another [1,2].

Diabetes mellitus (DM) is a clinical syndrome characterized by inappropriate hyperglycemia caused by the deficiency of insulin or by a resistance to the action of insulin at the

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cellular level [3]. It is a metabolic disorder of multiple etiologies characterized by insulin resistance, relative insulin deficiency and hyperglycemia with disturbances of carbohydrates, fat and protein metabolism [4]. The metabolic deregulations associated with DM causes secondary pathophysiological changes in multiple organ systems, which are associated with oxidative stress and damage to tissues [5].

Several drugs such as Biguanides and Sulfonylureas are presently available to reduce the hyperglycaemia for diabetes mellitus [6]. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because of these synthetic drugs have several limitations such as side effects, expensive, toxicity etc., [7]. Though so many known antidiabetic medicines are available in the market, remedies from the medicinal plants are used with success to this disease, because they are considered to be less toxic and free from side-effects when compared to synthetic one [8]. Management of diabetes with out any side effect is still a challenge to the modern medicine.

The traditional healers of *Malayali* tribes of Kalrayan (Kalvarayan) hills, Salem district are using varieties of plants as ethnomedicine. Among them *Waltheria indica* L. is used to cure DM. This angiosperm plant is belongs to Sterculiaceae family and it is an erect perennial herb. Leaves are stalked with shallow and irregularly toothed margins. Flowers are yellow and occurs in clusters [9]. It is distributed throughout India and occurs mostly in scrub forests of tropical and subtropical regions. Grows well in river banks, sandy clay or red soils and also in disturbed soils [10]. The medicinal properties of this plant are diverse. Apart from DM, this plant is used for the treatment of fever, syphilis, skin eruptions, wounds, cough, cold, vaginal infections, hypertension, ulcers, etc., world wide [11-14] and also acts as a potent antimicrobial [1, 15] and antiparasitic [16] agent. Hence, the present study is proposed for the scientific validation of the ethnomedicinal plant *Waltheria indica* L. used by the traditional healers of Kalrayan hills against DM with reference to its antidiabetic activity, and antioxidant properties, lipid profile and histopathology of liver and kidney tissues of the test animals.

2. MATERIALS AND METHODS

Collection and extraction of plant materials

The entire plant of *Waltheria indica* L. was freshly collected from "Kalrayan hills, Salem district, Tamil Nadu, India, lies between 11° 36’ and 12° 01’ N and 78° 29’ and 78° 54’ E, at an altitude of 1000 ft above MSL. The plant was identified with the help of standard local flora [9, 17] and further authenticated by taxonomists. The voucher specimens were submitted to the Department of Botany, Vinayaka Missions University, Salem, TN for further references. Plant materials are thoroughly washed using running tap water followed by rinsing with distilled water. The plant materials were then chopped, shade-dried at room temperature and coarsely powdered. The Soxhlet extraction procedure was carried out using 70% methanol. About 400 ml of the solvent was poured into the round bottom extraction flask and placed on the heating mantle top on which thimble containing 50g of the dried plant powder was placed. The condenser was placed above the thimble and the parts were fixed vertically. The extraction was carried out for 48 h. The extract was concentrated under reduced pressure using a rotary evaporator and was kept under refrigeration.

Preliminary phytochemical screening

Preliminary phytochemical screening was performed for the presence of alkaloids, carbohydrates, flavonoids, glycosides, oils and fats, polyphenols, tannins, terpens, saponins and triterpenoids for entire plant of *Waltheria indica* L. using standard qualitative assays [18, 19]

Experimental animal

The experiment was carried out using male Wistar albino rats (*Rattus norvegicus* / 6 weeks of age/ measuring 150–200 gm) procured from the Animal house, Nandha college of Pharmacy, Erode, Tamil Nadu, India. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (Regd no: 688/2/C-CPCSEA/2015) and are in accordance with the guidelines of the CPCSEA.

Housing conditions

The animals were housed in polypropylene cages and maintained under standard conditions (25± 2 ºC) and relative humidity of 30 – 70 % with 12 h dark/ light cycle. All animals were allowed to freely access to water and fed with standard commercial rat chow pellets (M/s. Hindustan Lever Ltd, Mumbai). The animals were fed with standard animal pellet diet and water *ad libitum.* Each animal was housed for 45 days.

Chemicals

Alloxan was obtained from Sigma – Aldrich Fine chemicals (St. Louis, MO, USA). Glibenclamide and other chemicals of analytical grade were purchased from local firms (India).

Antidiabetic properties of *Walthteria indica* L. in animal models

Experimental induction of diabetes

After fasting for 18h the animals were administered with single i.p. injection of freshly prepared alloxan solution (60mg/kg) in cold 0.1M citrate buffer (pH 4.5). After i.p. injection, the animals were allowed to free access to feed and water and provided with 5% glucose solution over night to counter the hypoglycemic shock. After two days of alloxan administration, rats with blood glucose concentration more than 250mg/dl were considered diabetic and were included in the study.

Experimental design

A total of 30 rats (6 normal; 24 diabetic) were used. The rats were divided into five groups of six each.

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Group I - Served as normal control animals, received 0.5 % Carboxy Methyl Cellulose (CMC) solution (1ml/kg PO) for 14 days.

Group II – Alloxan induced diabetic animals (Diabetic control), received 0.5 % CMC solution (1ml/kg PO).

Group III – Alloxan induced diabetic animals (Reference control), received the standard drug Glibenclamide 5mg/kg PO.

Group IV - Alloxan induced diabetic animals, received Methanolic extract of entire plant *Waltheria indica* 200mg/kg orally.

Group V - Alloxan induced diabetic animals received, Methanolic extract of entire plant *Waltheria indica* 400 mg/kg orally.

Changes in the level of glucose in control and experimental groups

The test drugs were administered orally once daily for 14 days by suspending in 0.5% CMC (Vehicle material) and Glibenclamide was used as standard drug [20]. All the group of animals received the treatment for 14 days. Blood samples were collected from tail 2 h after the drug administration on 0, 4th, 7th and 14th days to determine the blood glucose level by Glucometer (One-Touch).

Variations in the lipid profile of alloxan induced diabetic rats

On 15th day blood was collected in a non-heparinized tube by retro orbital sinus puncture, under phenobarbitone anaesthesia. Blood samples were collected in two different tubes (i.e) one with anticoagulant for plasma separation and another tube without anticoagulant for serum separation. Plasma and serum were obtained by centrifugation for 10 min at 2000 rpm and were used for various estimations. The separated plasma and serum were subjected to various biochemical tests like TC, TG, HDL-C, and LDL-C levels.

Total cholesterol

Total cholesterol in serum was determined by colorimetric method. The assay principle is based on enzymatic hydrolysis and oxidation of cholesterol. The indicator compound, quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The reagents consists of 4-aminoantipyrine (0.03 mmol/l), phenol (6 mmol/l), peroxidase (0.5 U/ml), cholesterol esterase (> 0.15 U/ml), cholesterol oxidase(> 0.1 U/ml) and Phosphate buffer (80 mmol/L pH 6.8). The serum sample (10 µl) was mixed with 1 ml of reagent, incubated at 37°C for 5 min and absorbance was measured at 500 nm against the reagent blank [21].

Triglycerols

Serum Triacylglycerols (TG) were determined by colorimetric method. The assay principle is based on the enzymatic hydrolysis of TG with lipases and the indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic activity of peroxidase [22]. The enzyme reagent consists of 4-aminophenazone (0.5 mmol/l), ATP 1.0 m.mol/l), lipases (150 U/ml), glycerol-kinase (0.4 U/ml), glycerol-3-phosphate oxidase (1.5 U/ml) and peroxidase (0.5 u/ml). The serum sample (10 µl) was mixed with 1000 µl of enzyme reagent, incubated at 37°C for 5 min and absorbance was measured at 500 nm against the reagent blank.

HDL Cholesterol

Serum HDL cholesterol was determined by colorimetric method. The assay principle is based on the following: the low density lipoproteins (LDL and VLDL) and chylomicron fraction is precipitated quantitatively by the addition of Phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined. The precipitation reagents consists of Phosphotungstic acid (0.55 mmol/l) and Magnesium chloride (25 mmol/l). The serum sample (200 µl) was mixed with 500 µl of precipitation reagent and centrifuged at 4000 rpm for 10 min. The supernatant (100 µl) was incubated at 37°C for 5 min and absorbance measured at 500 nm against the reagent blank [23].

LDL and VLDL Cholesterol

LDL and VLDL were calculated according to Friedwald formula [24].

LDL = TC – HDL – VLDL

VLDL cholesterol = Triglycerides / 5.

Preparation of tissues for histopathology and tissue homogenate for antioxidant study

At the end of the period, the rats were anaesthetized by intramuscular injection of Ketamine (90 mg / kg body weight) and Zylazine (10 mg / kg) and sacrificed by cervical decapitation. Liver and kidney were dissected out, washed with cold saline to clear the blood from the samples. A part of the liver and kidney tissues were immediately kept in ice-cold containers containing 10% formaldehyde for histopathological studies. The weighed quantity of sample tissues were homogenized in Potassium chloride (10mM), Phosphate buffer (1.15%), Ethylene-Diamine Tetra Acetic acid (EDTA; pH 7.4) and were centrifuged at 10,000 rpm for 60 min. The supernatant liquid was used for the assay of the antioxidant markers LPO, SOD, CAT, GPx and GSH. Carcasses of the animals were disposed by burial.

Estimation of lipid peroxidation of rat liver and kidney (LPO)

Lipid peroxidation in liver and kidney was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS) [25]. 0.1 ml of tissue homogenate was treated with 2 ml of TBA-trichloroacetic acid-HCl reagent (0.37% TBA, 0.25 M HCl and 15% TCA, 1:1:1 ratio), placed for 15 min in a water bath and then cooled and centrifuged at 3500 rpm for 10 min at room temperature. The absorbance of clear supernatant was measured at 535 nm against reference blank. Values were expressed as Mm/100 g tissue.

Estimation of Superoxide Dismutase (SOD)

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The activity of Superoxide dismutase (SOD) was carried out based on the oxidation of epinephrine adrenochrome transition by enzyme [26]. The post mitochondrial rat suspension (PMS) of rat tissue 0.5 ml was diluted with 0.5 ml distilled water. To this, chilled ethanol 0.25 ml and 0.15 ml of chloroform was added. The mixture was shaken for 1 min and centrifuged at 2000 rpm for 10 min. The PMS 0.5 ml was added with 1.5 ml phosphate buffer (pH 7.2). The reaction initiated by the addition of 0.4ml epinephrine and change in optical density OD was measured at 470 nm. SOD activity was expressed as U/mg of protein. Change in OD at 50% inhibition to adrenochrome transition by the enzyme was taken as one enzyme unit.

Estimation of Catalase (CAT)

The reaction mixture 1.5 ml vol contained 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Then the absorbance was measured at 530 nm; CAT activity was expressed as μM of H_2O_2 consumed/min/mg protein [27].

Estimation of Glutathione peroxidase (GPx)

The reaction mixture contained 0.2 ml of 0.4 M phosphate buffer (pH 7.0), 0.1 ml of 10 mM Sodium azide, 0.2 ml of tissue homogenized in 0.4M phosphate buffer (pH 7.0.), 0.2 ml of reduced glutathione and 0.1 ml of 0.2 mM H_2O_2. These contents were incubated for 10 min at 37°C and 0.4 ml of 10% TCA was added to stop the reaction and centrifuged at 3200 rpm for 20 min. The supernatant was assayed for glutathione content using Ellman's reagent (19.8 mg 5,5'-dithiobisnitrobenzoic acid [DTNB] in 100 ml 0.1% Sodium Nitrate). The activities were expressed as μg of GSH consumed/ min/mg protein [28].

Estimation of reduced glutathione(GSH)

The PMS of rat tissue (720 μl) and 5% TCA were mixed to precipitate the protein content of the supernatant. After centrifugation at 10,000 rpm for 5 min, the supernatant was collected. Ellman's reagent (DTNB (5, 5'-dithio-bis-2-nitrobenzoic acid) was added to it and the absorbance was measured at 412 nm [29].

Statistical Analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's 't' test using GraphPad version 3. $P<0.05$, $P<0.01$ and $P<0.001$ were considered as significant, more significant and most significant respectively.

Histopathological studies

Histopathological studies were carried to analyze the changes in the kidney and liver cell homogenates of the test animals [30]. The tissues were dissected out and washed in running tap water. Tissues were fixed by chemical fixation procedure using 10% aqueous solution of formaldehyde at neutral pH. It inactivates the enzyme reaction otherwise the tissue might be degraded. It is followed by dehydration and clearing process using alcohol (0% to 100%) and xylene. After clearing, the tissues were embedded in paraffin. Then tissue sections of 5-8μm thickness were taken using microtome and stained with Ehrlich Haematoxylein and counter stained with Eosin (H&E) stain [5, 31]. The excess stain was removed by washing the slide in running tap water and air dried. The tissue sections were observed under microscope and photomicrographs were taken.

3. RESULTS AND DISCUSSION

The phytochemical studies revealed the presence of various phytochemicals such as alkaloids, carbohydrates, flavonoids, glycosides, polyphenols, tannins, saponins and triterpenoids in *W. indica*. No oils and fats were detected in the test. It correlates with the results of previous studies [32].

The antidiabetic effect of methanolic extract of *W. indica* against alloxan induced diabetic rat was studied and the results were tabulated (Table 1). The blood glucose level was measured on 0, 4th, 7th and 14th day after drug treatment. In diabetic animal, a gradual increase in blood sugar level (BSL) was observed due to alloxan administration. The reference control Glibenclamide showed more decrease in BSL ($P<0.0001$) on 4th, 7th and 14th days. Diabetic rats treated with methanolic extract of the plant at both 200mg and 400mg/kg doses showed significant decrease in BSL at 4th, 7th and 14th days over diabetic control. When compared to the reference control, increased BSL was observed in both of the test groups. Among them, the results of groups treated with 400mg/kg of methanolic extract showed significant decrease in all the three samples.

Results of lipid parameters in alloxan induced diabetic rats showed a moderate decrease in total cholesterol, triglyceride, VLDL and LDL where as in HDL, significant increase was observed in both the test groups ($P<0.01$; $P<0.05$) over diabetic control (Table 2). When compared to the reference control, 400mg/kg dose showed significant results than 200mg/kg ($P<0.01$; $P<0.001$). The overall results of lipid parameters showed dose dependent effect.

Antioxidant properties of liver and kidney tissues showed significant results (Table 3 and Table 4). In liver homogenate of alloxan induced diabetic rats treated with test drug, SOD, CAT, GSH and GPx were significantly increased ($P<0.05$; $P<0.001$) where as LPO was significantly decreased when compared to the diabetic control ($P<0.01$). In kidney homogenate of alloxan induced diabetic rats, SOD, CAT, GSH and GPx ($P<0.05$; $P<0.01$; $P<0.001$) were significantly increased where as LPO was significantly decreased ($P<0.01$) over diabetic control. When compared to the reference control, the animals treated with 400mg/kg showed significant results than the animals treated with 200mg/kg of plant extract. The antioxidant properties of the plant also having dose dependent effect. It correlates with the previous findings [1, 33].

Results of histopathological studies of liver and kidney cells of alloxan induced diabetic animals showed significant

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healing property of the test drug. In diabetic control, the liver tissues showed hepatocytes, degeneration of central vein, congestion and cellular necrosis where as in the reference control rearrangement of normal hepatocytes with mild fatty degeneration and congestion was observed. Mild hepatic necrosis and congestion were observed in animals treated with 200mg/kg of *W. indica* whereas in animals treated with 400mg/kg of *W. indica* extract showed restored hepatic cell rearranged with mild congestion (fig1).

The T.S. of kidney cells showed well arranged cells with compact glomerular basement membrane with normal alternating areas of convoluted tubules, glomeruli and straight tubules in normal control animals. In diabetic control animals, congestion of convoluted tubules, disarranged glomeruli with glomerulosclerosis were observed. Mild thickening of glomerular basement with normal cell arrangement were observed in the reference control group. T.S of kidney tissues of the test groups showed mild congestion of tubules and necrosis of cells in animals treated with 200mg/kg dose whereas in animals treated with 400mg/kg of *W. indica* extract showed moderate congestion of tubules and mild necrosis of cells with clear glomeruli (fig2).

Alloxan induction paves the ways for the decreased utilization of glucose by the tissue and elevation of blood glucose level. Expression of elevated blood glucose level confirmed the development of diabetics in alloxan induced experimental animals [20]. It was evident from the results that untreated diabetic rats have elevated blood glucose level and the test drugs were able to correct the metabolic deviation from the diabetic control significantly.

Significant lowering of total cholesterol and rise in HDL cholesterol is a very desirable biochemical state for the prevention of hypoglycemia. Hence, the test drugs are supposed to have antidiabetic properties. The antioxidant activity could be the reason for therapeutic potential in preventing the development of oxidative stress involved diseases. The antioxidant properties of the plant is due to the presence of phenolic compounds. These phenolic compounds possess ideal structural chemistry for free radical scavenging activity.

The decrease in SOD, CAT, GSH were observed in the liver of diabetic control animal. SOD is ubiquitous cellular enzyme that dismutases super oxide radical to H_2O_2 and it is considered as one of the important cellular defensive mechanisms[34]. Catalase is an enzymatic antioxidant actively involved in red blood cells and liver extensively, spread in all animal tissues. This antioxidant decomposes H_2O_2 and protects the animal tissues from highly reactive hydroxyl free radicals[35]. Depletion of catalase observed in diabetic control group was found to be indicating the good antioxidant nature of the selected plants. The most important biomolecule against chemically induced toxicity is GSH which involves in elimination of reactive intermediates by reduction of hydroperoxides[36].

Polyphenolic substances such as flavonoids and tannins and various plant extracts exert antioxidant activities. Lipid peroxidation is a free radical chain reaction which is triggered by hydroxyl radical and leads to membrane break down and leading to produce more number of free radicals. The flavonoid components of plants extract known to be efficient in scavenging the highly reactive hydroxyl radical and superoxide onions and inhibit the lipid peroxidation by quenching the peroxyl radicals. Hence, flavonoids and polyphenolic compound might be responsible for the increase in SOD, catalase, GSH and thus lead to decrease in lipid peroxidation levels in alloxan induced diabetic rats.

Development of diabetes due to the administration of Alloxan is because of the generation of free radicals; a significant reduction in antioxidant enzyme levels is indicated as the potential reason for the susceptibility of organs to atrophy in diabetic states.

The phytochemicals present in the plants either singly or in combinations with each other could be responsible for the *in vitro* antidiabetic activity of the plant extracts. Recent studies have shown that phenolic phytochemicals have high antioxidant activity and certain therapeutic effect including antidiabetic activity[37.]

Numerous experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to an increased formation of free radicals and consequently to the onset of oxidative stress which has been implicated in diabetic complications. Oxidative stress is a condition of reduction in antioxidant enzymes like SOD, GSH and Catalase levels

From the observations, it was evident that treatment of diabetic rats with *W. indica* had not only show a significant antidiabetic activity but also possess an effective antioxidant properties, provides significant changes in the lipid profile and histopathology of the treated animals..

Table 1: Antidiabetic effect of methanolic extract of *Waltheria indica* in alloxan induced diabetic rats.

Groups	Drug Treatment	Blood Sugar Level (mg/dl)					
		Initial	After Alloxan	During Drug Treatment			
				0 Day	4th Day	7th Day	14th Day
I	Normal Control 0.5% CMC	101.67± 2.47	100.33± 4.40	98.00± 3.41	100.00± 2.50	98.50± 3.07	95.83± 2.81
II	Diabetic Control Alloxan (60mg/kg, i.p)	97.33± 2.81	267.17± 3.58	266.67± 3.03	262.33± 7.79	267.83± 5.26	270.50± 5.47
III	Diabetic +Reference Control Glibenclamide (5mg/kg)	96.00± 3.79	273.50± 5.37	278.50± 4.43	194.17± 3.09***	129.00± 2.57***	99.50± 1.77***
IV	Diabetic + *W.indica* (200mg/kg)	103.17± 4.62	271.50± 4.96	272.50± 4.54	236.00± 3.69*	168.33± 9.99***	155.83± 11.78***
V	Diabetic + *W.indica* (400mg/kg)	104.33± 4.23	265.33± 5.29	268.83± 3.32	214.67± 4.37***	141.50± 4.54***	119.00± 3.20***

Values are in Mean ±SEM (n=5)

*P<0.05, ** P<0.01 and** *P<0.001 Vs Diabetic Control

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Table 2. The effect of methanolic extract of *Waltheria indica* L. on lipid parameters in alloxan induced diabetic rats

Groups	Drug Treatment	Lipid Profile (mg/dl)				
		Total Cholesterol	Triglyceride	HDL	VLDL	LDL
I	Normal Control 0.5% CMC	65.30±2.01	52.08±1.52	32.32±1.03	10.42±0.31	22.57±2.26
II	Diabetic Control Alloxan (60mg/kg, i.p)	112.62±1.53	95.39±1.49	20.48±1.38	19.07±0.29	73.07±0.48
III	Diabetic +Reference Control Glibenclamide (5mg/kg)	87.26±2.62**	71.42±1.37**	28.39±1.22**	14.28±0.27**	44.58±1.34***
IV	Diabetic + *W. indica* (200mg/kg)	93.35± 2.39*	74.47±1.64**	27.02±1.35**	14.89±0.33**	50.88±2.62**
V	Diabetic + *W. indica* (400mg/kg)	79.45±1.59**	69.53±1.36**	29.40±1.26***	13.90±0.27**	36.16±3.02***

The values were expressed as Mean ± SEM (n=6)
*P<0.05, **P<0.01 & ***P<0.001 Vs Diabetic Control

Table 3: Antioxidant effect of methanolic extract of *Waltheria indica* L. in liver homogenate of alloxan induced diabetic rats

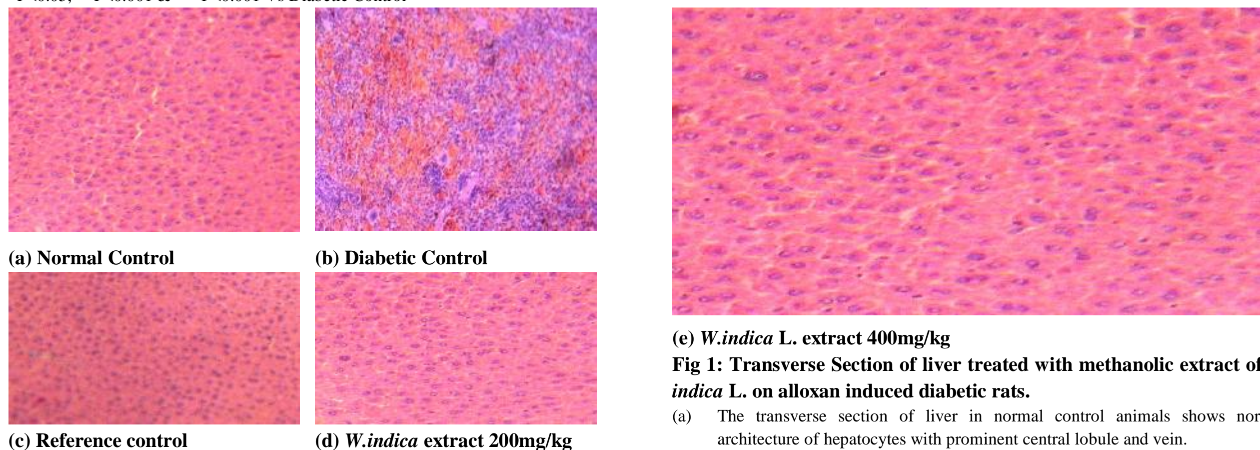
Groups	Drug Treatment	Liver Homogenate				
		LPO Mm/100 g of Tissue	SOD U/mg of Protein	CAT µM of H_2O_2 consumed/min/mg protein	GSH µg of GSH consumed/min/mg protein	GPx µg of GSH utilized/min/mg protein
I	Normal Control 0.5% CMC	0.23± 0.02	1.46±0.04	0.97±0.03	0.69±0.07	0.98±0.09
II	Diabetic Control Alloxan (60mg/kg, i.p)	0.56± 0.01	0.88±0.04	0.54±0.06	0.35±0.053	0.49±0.04
III	Diabetic +Reference Control Glibenclamide (5mg/kg)	0.29± 0.02***	1.31±0.06***	0.84± 0.04***	0.54±0.06	0.89± 0.04***
IV	Diabetic + *W. indica* (200mg/kg)	0.31± 0.02**	1.09±0.09^{n*}	0.80± 0.05***	0.49±0.06*	0.75± 0.05*
V	Diabetic + *W. indica* (400mg/kg)	0.26± 0.01***	1.18±0.10*	0.91± 0.06***	0.54±0.06*	0.89± 0.09***

The values were expressed as Mean ± SEM (n=6)
*P<0.05, **P<0.001 & ***P<0.001 Vs Diabetic Control

Table 4: Antioxidant effect of methanolic extract of *Waltheria indica* L. in kidney homogenate of alloxan induced diabetic rats

Groups	Drug Treatment	Kidney Homogenate				
		LPO Mm/100 g of Tissue	SOD U/mg of Protein	CAT µM of H_2O_2 consumed/min/mg protein	GSH µg of GSH consumed/min/mg protein	GPx µg of GSH utilized/min/mg protein
I	Normal Control 0.5% CMC	0.26± 0.03	0.75±0.02	1.24±0.11	0.68±0.09	0.84±0.08
II	Diabetic Control Alloxan (60mg/kg, i.p)	0.56± 0.05	0.37±0.04	0.67±0.07	0.28±0.07	0.38±0.06
III	Diabetic +Reference Control Glibenclamide (5mg/kg)	0.33± 0.05*	0.71±0.05***	1.07±0.09*	0.59±0.09**	0.76±0.11**
IV	Diabetic + *W. indica* (200mg/kg)	0.36± 0.05*	0.59±0.05**	0.83±0.04*	0.45±0.03 *	0.63±0.08*
V	Diabetic + *W. indica* (400mg/kg)	0.33± 0.07*	0.69±0.04***	0.96±0.10 *	0.52±0.09 *	0.76±0.08**

The values were expressed as Mean ± SEM (n=6)
*P<0.05, **P<0.001 & ***P<0.001 Vs Diabetic Control



(a) Normal Control **(b) Diabetic Control**

(c) Reference control **(d) *W.indica* extract 200mg/kg**

(e) *W.indica* L. extract 400mg/kg

Fig 1: Transverse Section of liver treated with methanolic extract of *W indica* L. on alloxan induced diabetic rats.

(a) The transverse section of liver in normal control animals shows normal architecture of hepatocytes with prominent central lobule and vein.

(b) The TS of liver in diabetic control shows destruction of hepatocytes, degeneration of central vein, congestion and cellular necrosis.

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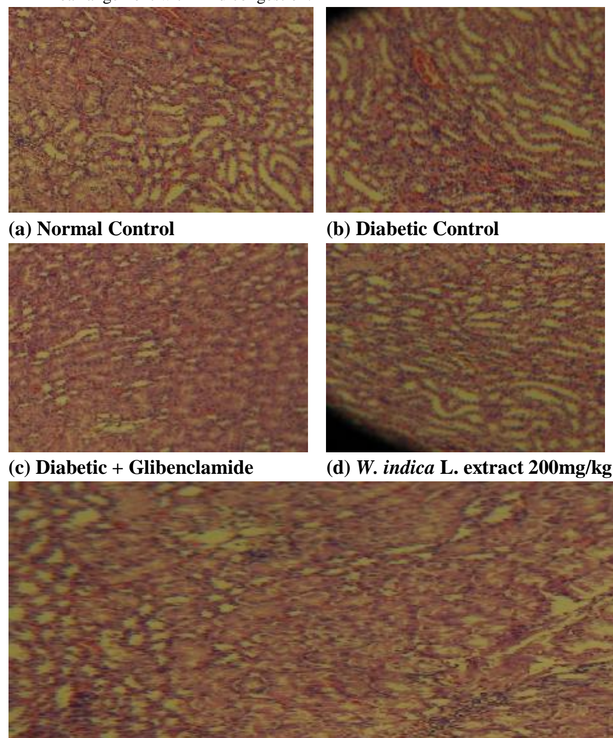
(c) The TS of liver in reference control shows rearranged normal hepatocytes with mild fatty degeneration and congestion.

(d) The section of liver treated with 200mg/kg of *W indica* shows mild hepatic necrosis and congestion.

(e) The section of liver treated 400mg/kg of *W indica* shows restored hepatic cell rearrangement with mild congestion.



(a) Normal Control **(b) Diabetic Control**

(c) Diabetic + Glibenclamide **(d) *W. indica* L. extract 200mg/kg**

(e) *W. indica* L. extract 400mg/kg

Fig 2: Transverse Section of Kidney treated with methanolic extract of *W indica* L. on alloxan induced diabetic rats

(a) The transverse section of kidney in normal control animals shows well arranged cells with compact glomerular basement membrane. The section also shows normal alternating areas of convoluted tubules, glomeruli and straight tubules.

(b) The kidney in diabetic control animals showed congestion of convoluted tubules, dearranged glomeruli with glomerulosclerosis.

(c) The section of reference control shows mild thickening of glomerular basement with normal cell arrangement.

(d) The section of kidney treated 200mg/kg of *W indica* shows mild congestion of tubules and necrosis of cells.

(e) The section of kidney treated 400mg/kg of *W indica* shows the moderate congestion of tubules and mild necrosis of cells with clear glomeruli.

4. CONCLUSION

Present study on the antidiabetic properties of the ethnomedicinal plant *Waltheria indica* L. on the alloxan induced diabetic models provides the evidence to the scientific validation of the herbal plant used by the traditional healers of the Kalrayan hills, Salem against diabetics. Further it is supported by its effect on the lipid profile, antioxidant activity and histopathology of liver and kidney tissues of the treated animal models.

Treatment with methonolic extracts of *Waltheria indica* improved the glucose tolerance in alloxan induced diabetic rats significantly. The antioxidant properties of methonolic extracts has dose dependant effect. The overall assessment of histopathological studies revealed that the cells of liver and kidney tissues of alloxon induced diabetic animals were recovered from the tissue damage due to the drug administration. Further investigations are needed to elucidate the active constituents present in the extracts responsible for the antidiabetic property.

5. ACKNOWLEDGEMENTS

One of the authors (MK) is thankful to The Chancellor, Vinayaka Missions University, Salem and The Management, Nandha Group of Educational Institutions, Erode, for providing the facilities to carry out the research. The authors also grateful to Dr. R. Prabakaran, Department of Botany, Vivekanandha College of Arts and Sciences (W), Tiruchengode for identification of research plant. Authors like to thank Dr. T. Sivakumar**,** Principal, Nandha College of Pharmacy, Erode for granting permission to conduct the research in Nandha College of Pharmacy and Dr. S. Sengottuvelu**,** Department of Pharmacology, Nandha College of Pharmacy, Erode for his suggestions, all time assistance and helps rendered for the successful completion of this study. We express our gratitude to the traditional healers of the study area who has shared their valuable knowledge on medicinal plants.

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Conflict of Interest: None
Source of Funding: Nil

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