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## **ANTIDIABETIC, ANTIHYPERLIPIDAEMIC AND HEPATOPROTECTIVE ACTIVITY OF WHOLE PLANT ETHANOLIC EXTRACT OF *BLEPHARIS REPENS* (VAHL) ROTH IN NORMAL AND ALLOXAN INDUCED DIABETIC RATS**

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### **ABSTRACT**

*Blepharis repens* (vahl) Roth belongs to family acanthaceae is a herb distributed throughout India, Srilanka and Africa. The aim of the present study was to evaluate the antidiabetic potential of whole plant ethanolic extract of *Blepharis repens* (vahl) Roth in normal and alloxan induced diabetic rats. The Preliminary phytochemical screening shows the presence of carbohydrates, Alkaloids, phytosteroids, flavonoids, tannins, Saponins, fixed oils. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg, i.p). The Whole plant ethanol extract of *Blepharis repens* (vahl) Roth at a dose of 100 and 200mg/kg of body weight was administered at single dose per day to diabetes induced rats for a period of 21 days. The effect whole plant of ethanol extract of *Blepharis repens* (vahl) Roth on blood glucose, serum lipid profile [total cholesterol (TC), triglycerides (TG), high density lipoprotein – cholesterol (HDL-C), low density lipoprotein – cholesterol (LDL-C), very low density lipoprotein – cholesterol (VLDL-C) and serum protein, albumin, globulin, serum enzymes [serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transaminases (SGOT), alkaline phosphatase (ALP)], were measured in the diabetic rats. The Whole plant ethanol extract of *Blepharis repens* (Vahl) Roth elicited significant reductions of blood glucose ( $P < 0.05$ ), lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C at the dose of 200mg/kg was compared with the standard drug Glibenclamide 2.5gm/kg). From the above results, it is concluded that ethanol extract of whole plant *Blepharis repens* (vahl) Roth possesses significant antidiabetic, antihyperlipidaemic and hepatoprotective effects in alloxan induced diabetic rats.

**Key Words:** Antidiabetic, antihyperlipidaemic and hepatoprotective activity, WEEBR- Ethanolic extract of *Whole plant Blepharis repens* (vahl) Roth, Alloxan induced Diabetic rats.

### **INTRODUCTION**

Diabetes mellitus, a chronic metabolic disorder,

has now become an epidemic, with a worldwide incidence of 5% in general population. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS (Edwin JE *et al.*, 2008). Plants have been the major source of drugs for the treatment of diabetes mellitus (DM) in Indian medicine and other ancient systems in the world, and for a long time DM

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has been treated orally with herbal medicines or their extracts (Akhtar FM and Ali MR, 1984), because plant products are frequently considered to be less toxic and more free from side effects than synthetic ones (Brinker F, 1998). Furthermore, after the recommendations made by the WHO on DM, investigations on hypoglycaemic agents from medicinal plants have become more important and the search for more effective and safer hypoglycaemic agents has continued to be an important area of active research. World ethno botanical information about medicinal plants reports that almost 800 plants could be used to control DM. Many herbs and plants have been described as possessing hypoglycaemic activity when taken orally (Pepato MT *et al.*, 2003). *Blepharis repens (vahl) Roth* belongs to family acanthaceae is a large sized plant distributed throughout India, Srilanka and Africa. Flavonoids, Alkaloids, phenol, saponins and essential minerals with good nutritive value and secondary metabolites (Khare CP, 2007). The plant used as Diuretics, Antidiarrhoeal, Antidote for snake bite, Anti-hypertension, Analgesic & antipyretic, Anthelmintic, joint pains, muscle strains, abortifacient.

The root part used against kidney diseases and whooping cough. Apart from this, plant parts used in gonorrhoea, syphilis, bladder stones, bronchitis and cancer, heart ailments, colds, fever, hypertensions, and stomach problems (Timothy). The objective of the present work to evaluate the antidiabetic, antihyperlipidaemic and hepatoprotective activity of *Blepharis repens (vahl) Roth* by using animal models.

## MATERIAL AND METHODS

### Chemical

Alloxan were obtained from Sigma Chemical Co (St Louis, MO-USA). Bio-chemical kits and all other chemicals utilized were of analytical grade.

### Plant Material

The plant *Blepharis repens (vahl) Roth* were collected from an open field around Western Ghats of Gobichettipalayam, Erode District, Tamilnadu. Dr.P.Jayaram Ph.D carried out identification of the plant at the National Institute of Herbal Science (PARC) Chennai.

### Preparation of plant extract

The Whole plant *Blepharis repens (vahl) Roth* of were first washed several times with distilled water and dried well. The Whole plant was dried at room temperature and coarsely powdered. The powder was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with petroleum ether, chloroform, ethyl acetate, ethanol using Soxhlet apparatus (Vinod DR, 2002).

### Preliminary phytochemical screening

One gram of the petroleum ether, chloroform, ethyl acetate, ethanol extracts of *Blepharis repens (vahl) Roth* were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration 1% (v/v). The extracts thus obtained were subjected to preliminary phytochemical screening (Vinod DR, 2002; Kokate CK, 2007; Mukherjee PK, 2002; Harborne JB, 1998).

### Animals

The study was conducted on forty matured Wistar strain male albino rats; 3 months of age weighing about 150-200 g (Daisy P *et al.*, 2009). Animals were acclimated for a period of fifteen days in our laboratory conditions prior to the experiment. Rats were housed in tarsons cages (six rats per cage), at an ambient temperature of  $25 \pm 20^\circ\text{C}$  with 12 h light: 12 h dark cycle. Rats have free access to standard food and water *ad libitum*. The Principles of Laboratory Animal Care (NIH, 1985) were followed throughout the duration of experiment and instruction given by our institutional ethical committee was followed regarding injection and other treatment of the experiment. Normoglycemic animals were selected for this experiment having the fasting blood glucose level of  $75 \pm 5$  mg/dl.

### Acute toxicity studies

The acute toxicity of ethanolic extracts of *Blepharis repens (vahl) Roth* were determined by using female albino Wistar rats (150-200 g) which were maintained under the standard conditions. The animals (n=5 per dose) were fasted 12 h prior to the experiment, up and down procedures were adopted for toxicity studies. Animals were administered with single dose of extract of *Blepharis repens (vahl) Roth* leaves at a dose of 2000 mg/kg and observed for their mortality during 2 and 7 days study period (short term) toxicity and the dose increased up to 5000 mg/kg and were observed up to 7 days for their behavioural, economical and neurological profiles except slight depression in their activity (Ecobichon DJ, 1997).

### Induction of Diabetes Mellitus

Male Wistar strains of rats, each weighing 150-200 g were used for the study. They were housed in polypropylene cages lined with husk, renewed every 24 h under 12/12 h light/dark cycles at  $25-30^\circ\text{C}$  and at 45%–55% relative humidity. The animals were fed with a standard rat pellet diet and tap water was supplied *ad libitum*. A freshly prepared solution of alloxan monohydrate (120mg/kg body weight), in sterile normal saline solution, was injected intraperitoneally to overnight fasted rats (Demerdash FM *et al.*, 2005). Blood glucose was measured after 72 hours of alloxanisation by one-touch glucometer, and it was confirmed by testing for glucosuria using glucose indicator sticks. Rats showing fasting blood glucose (FBG) levels  $> 250$  mg/dL were selected as diabetic in this experiment.

### Experimental design

Diabetes was induced in rats within 48 hours by the intra peritoneal administration of alloxan dissolved in distilled water (5%) in a dose of 100mg/kg body weight. The rats were divided into 5 groups of 6 animals each.

**Group I:** (Untreated Control): Normal control received only saline (10ml/Kg),

**Groups II:** (Diabetic Control): Diabetic control, received alloxan and saline,

**Groups III and IV:** (Diabetic + WEEBR): Received alloxan and 48 hours later they were treated orally with ethanolic extract of Whole plant *Blepharis repens* (Vahl) Roth at doses of 100 and 200mg/kg,

**Group V:** (Diabetic + glibenclamide): Was treated with Glibenclamide (2.5mg/kg) (Jaouhari JT *et al.*, 2000) as Standard. All the group of animals received the treatment by the above schedule for 21 days.

### Antidiabetic activity

The fasting blood glucose levels (FBGL) of all the rats were recorded at regular intervals during the experimental period (0 day, 1st week and 2nd week, 3<sup>rd</sup> week). Blood samples were collected by tail vein and FBG level were measured by single touch glucometer. From all groups of rats (normal, diabetic control, extracts and standard treated) and mild Anaesthesia where was separated by centrifugation of sample at 4000rpm for 10mins and stored in the refrigerator until analysed. The serum was subjected for the estimation of Triglycerides (TGL, HDL, LDL, VLDL and total cholesterol level. Results were analyzed by the student test. The minimum level of significance was fixed at  $p < 0.01$ .

All the animals were sacrificed on 21<sup>st</sup> day by cervical dislocation, pancreas was excised/isolated and was subjected to histopathological studies and microscopical findings were noted.

### Hypolipidaemic activity

After 21 days of treatments (24 hours after the last dose), the animals were anaesthetized with ethyl vapour and the blood collected through cardiac puncture into sample bottles devoid of anticoagulant. The samples were centrifuged at 1000rpm for 15 minutes to obtain the sera. Serum cholesterol, triglyceride and high density lipoprotein (HDL) levels were measured by enzymatic colorimetric methods using Randox diagnostic kits. All samples were analyzed with a wine light Unicam spectrophotometer. The concentrations of low density lipoprotein (LDL) and very low density lipoproteins (VLDL) were calculated from the formula of Friedwald (Friedewald WT *et al.*, 1972).

### Hepatoprotective activity

After 21 days of treatments (24 hours after the last dose), the animals were anaesthetized with ethyl vapour and the blood collected through cardiac puncture into

sample bottles devoid of anticoagulant. The samples were centrifuged at 1000rpm for 15 minutes to obtain the sera. 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. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong.

### Statistical Analysis

Analysis of Variance (ANOVA) followed by Multiple comparison student two-tail 't' test was used for statistical analysis of collected data. Differences were considered significant at  $p < 0.05$ . All the values were indicated in the tables and figures as Mean  $\pm$  SEM.

## RESULTS

### Effect of EE of *Blepharis repens* (vahl) Roth Phytochemical Study

The plant *Blepharis repens* (vahl) Roth was extracted with various solvent by using Soxhlet apparatus. The percentage yields were 1.24% in petroleum ether, 1.11% in chloroform, 2.1% in ethyl acetate and 5.3% in ethanol and 4.5% w/w in table 1.

The phytochemical screening was done by the all extracts of *Blepharis repens* (vahl) Roth showed the presence of carbohydrates, Alkaloids, phytosteroids, flavonoids, tannins, Saponins, fixed oils.

### Effect of EE of *Blepharis repens* (vahl) Roth acute toxicity study

Acute toxicity studies revealed the non-toxic nature of the EE of *Blepharis repens* (vahl) Roth. There was no lethality or toxic reaction found at any of the doses selected until the end of the study period. All the animals were alive, healthy and active during the observation period.

### Effect of EE of *Blepharis repens* (vahl) Roth on fasting blood glucose level

The effect of oral administration of EE of *Blepharis repens* (vahl) Roth shows in table 3 in the level of fasting blood glucose level in normal and diabetic rats. There was a significant elevation in blood glucose in alloxan induced diabetic rats (Group II) when compared with normal rats (Group I). Administration of EE of *Blepharis repens* (vahl) Roth (Group III&IV) and glibenclamide (Group V) tends to bring the parameters significantly towards the normal.

### Effect of EE of *Blepharis repens* (Vahl) Roth on histopathological studies

Multiple section of pancreas were taken & studied for any histological changes. The pancreas present in the animal treated with WEEBR Extract showed normal

appearance of pancreatic lobules, Acini & cells. The islets were normal in size, shape & number comparatively similar to that of control. The pancreas of Alloxan Induced Diabetic rats showed congestion of pancreatic cells.

#### Effect of EE of *Blepharis repens (vahl) Roth* on hypolipidemic profile

The effect of oral administration of EE of

*Blepharis repens (vahl) Roth* shows table 4 in the levels of TC, TG, HDL-C, LDL-C and VLDL-C in the serum of normal and diabetic rats. The diabetic rats had elevated levels of serum TC, TG, LDL-C, VLDL-C and decreased level of HDL-C as compared with normal control rats. Diabetic rats treated with EE of *Blepharis repens (vahl) Roth* extract and glibenclamide reversed serum lipid profiles to near normal levels.

**Table 1. Extraction values of different extract of *Blepharis repens (vahl) Roth* Extracts % Yield (w/w)**

Extractives	<i>Blepharis repens (vahl) Roth</i> Extracts % Yield (w/w)
Petroleum ether	1.24
Chloroform	1.11
Ethyl acetate	2.1
Ethanol	5.3
Water	4.5

**Table 2. Qualitative phytochemical analysis of different extracts of *Blepharis repens (vahl) Roth* Particulars**

Extract	Alkaloids	Carbohydrates	Glycosides	Flavonoids	Proteins & Amino acids	Steroids	Tannins & Phenolic	Saponins	Fixed Oils
Petroleum ether	-	-	+	+	-	+	+	+	+
Chloroform	-	+	+	+	-	+	+	-	+
Ethyl acetate	-	-	+	+	-	+	+	-	-
Ethanol	-	+	+	+	-	-	+	-	-
Aqueous	+	+	-	+	-	-	+	+	+

(+) = indicates present; (-) = indicates absence.

**Table 3. Effect of ethanol extract of *Blepharis repens (vahl) Roth* on blood glucose level of normal, diabetic induced and drug treated rats at different week intervals Treatment Blood glucose (mg/dl)**

Groups	0 day(mg/ml)	After 7 days(mg/ml)	After 14 days(mg/ml)	After 21 days(mg/ml)
Normal control	104.5±3.50	103.7±1.20	103.5±1.16	101±1.01
Diabetic control	429.8 ± 3.01	405.0 ± 8.81	407.0 ± 6.61	408.0± 10.10
Glibenclamide(2.5mg/kg)	390.4 ± 4.50*	160.2 ± 2.90*	120.0 ± 2.15*	90.0 ± 2.05*
EEBR(100mg/kg)	393.6 ± 4.89*	165.2 ± 2.96*	159.4 ± 2.87*	145.4 ± 2.87*
EEBR(200mg/kg)	406.8 ± 4.51*	136.4 ± 3.30*	131.4 ± 3.27*	121.4 ± 3.25*

Values are given as mean ± SD for groups of six animals each. Values are statistically significant \*P<0.05.

Diabetic rats were compared with control rats; EEBR diabetic rats were compared with diabetic rats; glibenclamide treated diabetic rats were compared with diabetic rats

**Table 4. Effect of ethanol extract of *Blepharis repens (vahl) Roth* on the protein, albumin, globulin, SGPT, SGOT and ALP level of normal, diabetic induced and drug treated rats**

Treatment	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGOT (u/l)	SGPT (u/l)	ALP (u/l)
Normal control	6.67 ± 0.103	3.12± 0.10	2.75±0.07	62.32± 6.25	44.33± 6.90	167.0± 27.10
Diabetic control	5.67 ± 0.24*	2.12± 0.10 *	2.36±0.10*	122.67 ± 3.93*	72.00±5.86*	286.0± 10.21*
Diabetic+EEBR(100mg/kg)	5.82± 0.10*	2.96± 0.50*	2.62± 0.08*	81.33± 6.89*	61.67 ± 6.44 *	211.5±52.45 *
Diabetic+EEBR(200mg/kg)	6.05± 0.42*	3.10± 0.09 *	2.70± 0.041*	71.67± 8.81*	45.00±5.77 *	199.3± 29.45*
Glibenclamide(2.5mg/kg)	6.35± 0.25*	3.11± 0.18 *	2.72± 0.04*	68.00± 8.50 *	42.67± 3.93*	172.6. ± 19.20*

Values are given as mean ± SD for groups of six animals each. Values are statistically significant \*P<0.05.

Diabetic rats were compared with control rats; EEBR diabetic rats were compared with diabetic rats; glibenclamide treated diabetic rats were compared with diabetic rats

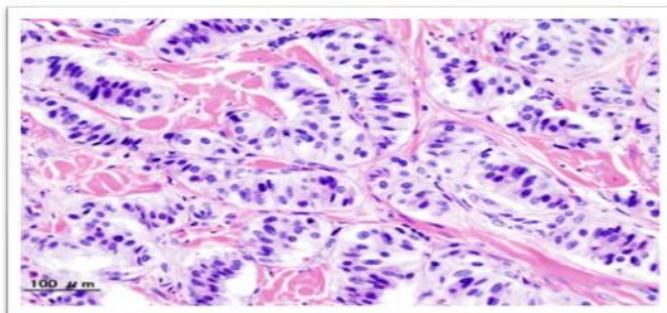
**Table 5. Effect of ethanol extract of *Blepharis repens (vahl) Roth* on the TC, TG, HDL-C, LDL-C and VLDL-C in the plasma of normal, diabetic induced and drug treated rats**

Treatment	TC(u/l)	TGL(u/l)	HDL-C(u/l)	LDL-C(u/l)	VLDL-C(u/l)
control	67.33± 4.63	98.66± 2.33	63.33± 4.56	44.33± 8.89	22.08± 7.15
Diabetic control	178.67± 4.67*	185.67± 12.5*	32.67± 3.92*	92.00± 6.86*	47.00± 10.21*
Diabetic+EEBR (100mg/kg)	91.33± 6.89*	113.67± 4.70*	41.33± 6.89*	31.67± 4.44*	19.06± 9.46*
Diabetic+ EEBR (200mg/kg)	77.67± 1.45*	110.33± 7.31*	53.67± 6.81*	40.00± 5.77*	20.75± 8.45*
Glibenclamide (2.5mg/kg)	70.67± 3.48*	109.33± 10.97*	61.00± 7.50*	42.67± 4.73*	21.60± 9.20*

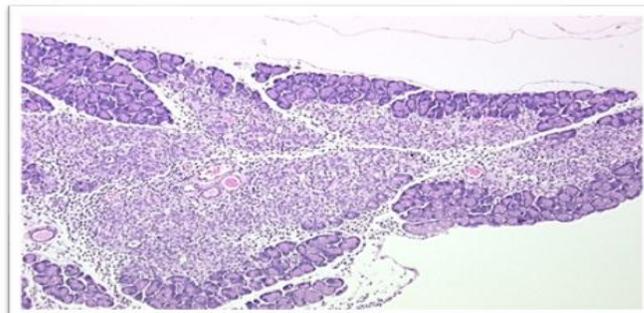
Values are given as mean ± SD for groups of six animals each. Values are statistically significant \*P<0.05.

#### NORMAL TRANSVERSE SECTION OF PANCREAS

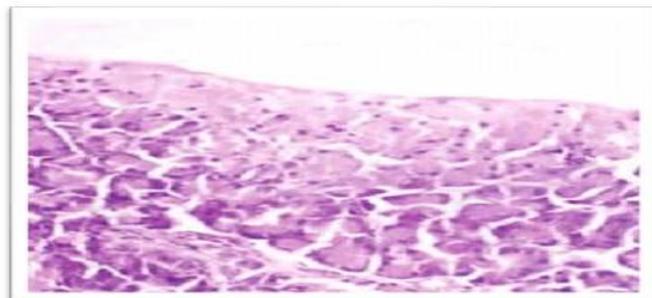
**Figure 1. Normal transverse section of pancreas.**



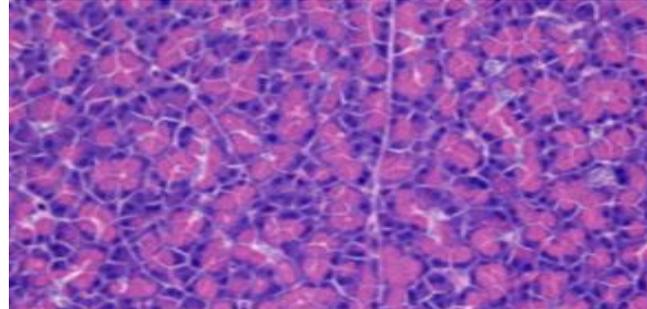
**Figure 2 Pancreas with alloxan Induced Diabetes**



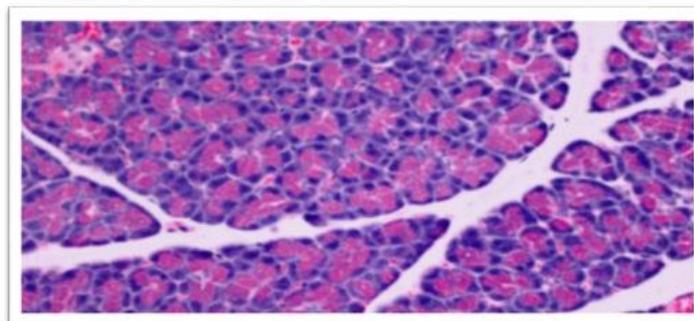
**Figure 3. Transverse Section of Pancreas with *Blepharis repens (Vahl) Roth*-100mg/kg**



**Figure 4. Transverse Section of Pancreas with *Blepharis repens (vahl) roth* Extract-200mg/kg**



**Figure 5. Transverse Section of Pancreas with Glibenclamide 2.5mg/kg**



#### **Effect of EE of *Blepharis repens (vahl) Roth* on hepatoprotective profile**

The effect of oral administration of EE of *Blepharis repens (vahl) Roth* shows in table 5 in the levels of total protein, albumin, globulin, and liver marker enzymes such as SGPT, SGOT and ALP in the serum of

diabetic rats. The diabetic rats (Group II) had decreased levels of serum total protein, albumin, globulin and elevated level of liver marker enzymes such as SGPT, SGOT and ALP when compared with normal control rats (Group I). After treatment with EE of *Blepharis repens (vahl) Roth* extract, glibenclamide, total protein, albumin,

globulin, and liver marker enzymes were brought back to near normal levels (Group III & IV&V).

## DISCUSSION

The present study is assessment of anti-hyperglycemic of EE of *Blepharis repens (vahl) Roth* on male wistar rats. Alloxan causes a significant elevation in the level of blood glucose in rats. Administration of 100 and 200 mg/kg body weight of EE of *Blepharis repens (vahl) Roth* significantly decreased the blood glucose level after twenty one days of treatment in these rats suggesting that it has hypoglycemic properties and also the hypolipidemic and hepatoprotective profile were restored to control levels with the administration of the known drug glibenclamide and Whole plant extracts of *Blepharis repens (vahl) Roth*. The result from the present study shows the significant changes in biochemical parameter during the experimentally induced diabetes.

### Histopathological Studies

*All the animals were Sacrificed on 21 st Day by Cervical Dislocation; Pancreas were excised, Isolated & were subjected to Histopathological Studies & Microscopical Finds were Noted.*

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 V). On administration of ethanol extract of *Blepharis repens (vahl) Roth* to the diabetic rats, protein, albumin and globulin levels were found to be restored in normal. These results were in accordance with the effect of *Wattakka volubilis* leaf in diabetic rats (King EJ, Armstrong AR, 1934). The animals treated with alloxan developed hepatic damage which was evident from the increase in the enzyme activities. Pretreatment with ethanol extract of *Blepharis repens (vahl) Roth* and glibenclamide resulted in a decrease of transaminase activities in alloxan treated rats. The serum AST and ALT levels increases as a result of metabolic changes in the liver, such as administration of toxin, cirrhosis of the liver, hepatitis and liver cancer including diabetes.

Similarly in the present study, it was observed that 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 alloxan. AST and ALT were used as markers to assess the extent of Liver damage in Alloxan induced diabetic rats. In this study, the whole plant ethanol extracts of *Blepharis repens (vahl) Roth* regulated the activity of SGPT, SGOT and ALP 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.

Alloxan induced diabetic rats showed significantly increased serum lipid profiles except HDL-C, when compared with normal rats. The glibenclamide and ethanol extract of *Blepharis repens (vahl) Roth* 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 with normal rats. On administration of ethanolic extract of *Blepharis repens (vahl) Roth* 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 a risk factor for coronary heart diseases. Lowering the serum lipid level through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease. It is concluded that, medicinal plants have been reported to possess antihyperglycemic activity.

*Blepharis repens (vahl) Roth* is gaining much importance in diabetic control as it has been used as a traditional medicine for diabetes; since the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, phytosteroids, tannins and Saponins, Alkaloids, Fixed oil. Several authors reported that flavonoids, steroids, terpenoids, phenolic acids are known to be bioactive antidiabetic principles. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues. Saponin reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intraluminal physicochemical reaction. Hence, it has been reported to have hypocholesterolemic effect and thus may aid a lessening metabolic burden that would have been placed in the Organ.

In the present study, the phytochemical analysis of ethanol extract of *Blepharis repens (vahl) Roth* clearly points out the presence of above said active principles. The preliminary investigation on the antidiabetic efficacy of ethanol extract of *Blepharis repens (vahl) Roth* will be significant to proceed further in this path for the isolation of active principles responsible for antidiabetic activity. Diabetic rats were compared with control rats; WEEBR diabetic rats were compared with diabetic rats; glibenclamide treated diabetic rats were compared with diabetic rats

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