In-vivo Screening of Ethanolic Extract of *Antigonon leptopus* Flower for Anti-diabetic and Antioxidant Potential

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ABSTRACT

Objectives: Medicinal plants have always been a consummate source for drugs and already provided us with lead compounds for numerous currently available drugs with no or less side effects. This supports the usage of herbal drugs for the treatment and management of various diseases. However, an assessment with available techniques has become an essential aspect to establish a valid scientific rationale for their usage in major healthcare problems. Methods: Ethanolic extract of Antigonon leptopus flower was screened for antioxidant activity by DPPH method and its effects on blood glucose levels (OGTT, acute and sub-acute study) in Alloxan induced diabetic rats, effects on serum lipid profiles and histopathological study of pancreas. Results: The extract showed potent in-vitro antioxidant activity. Treatment with ethanolic extract of Antigonon leptopus exhibits remarkable reduction in fasting blood glucose level (OGTT) at 2nd and 4th hr with a percentage of 26% and 11% respectively, whereas in acute study (at 6 hr) it was found to be 16 % and in sub acute study (15 days) it was reported to be 54%. Also, a significant lipid lowering activity was observed. Conclusion: This potent antioxidant, hypoglycemic and hypolipidemic efficacy may be attributed to the secondary metabolites (phenols, flavonoids, terpenoids, alkaloids, phytosterols and proteins) present. Key words: Antigonon leptopus, Anti-diabetic, Antioxidant, Hypolipidemic, DPPH.

INTRODUCTION

The medical and societal health concern of diabetes mellitus (DM) is more and increasing worldwide. All over the world, approximately 1.5 million deaths are occurring due to diabetes. Diabetes (Hyperglycemia) is classified as insulin dependent diabetes mellitus (IDDM or Type 1) and non-insulin dependent diabetes mellitus (NIDDM or Type 2). Type 1 DM is an autoimmune disorder characterized by T-cell mediated destruction of β -cells (pancreatic) and substantial lack in insulin production; whereas deficient secretion of; and resistance to insulin, both the factors are remarkably observed in more prevalent Type-2 DM.

Observance of polyurea (frequent urination), polyphagia (increased appetite or craving for food) and polydipsia (excessive thirst) characteristic symptoms are the of hyperglycemia.¹ DM is observed to be a set of metabolic malfunction, genetic and autoimmune disorder determined by a characteristic factor i. e. hyperglycemia. Metabolic aspects are outcome of partial or complete insulin deficiency.² The DM epidemiology exhibits dissimilarity in both types according to sex, age, racial factor and urban-rural population due to their lifestyles. A remarkable development has been made in understanding the disease so as to treat the causative factors of DM

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and to take preventive measures.³ Hyperglycemia; a chronic metabolic disorder is the outcome of hereditary and environmental aspects. Anomalous secretion of insulin; receptors or events occurring after receptor interactions are characteristics of DM resulting in malfunctioning of carbohydrate, protein and fat metabolism and electrolyte imbalance.⁴ This contributes to uncontrolled hyperglycemia, which significantly causes damage to kidney (nephropathy), cardiac diseases and poor vision (retinopathy); leading to morbidity and mortality. Increased blood glucose levels for longer duration in patients with DM are prone to develop varied vascular complications attributed to increased production of free radical (ROS-Reactive Oxygen Species) which suppresses activity of endogenous antioxidants; SOD (superoxide dismutase) and CAT (catalase).⁵ These free radicals are the outcome of several metabolic events taking place in our body, which must be scavenged effectively with body's cellular constituents to get the protection from developments of numerous disease conditions.6 The ROS initiated oxidation results in disintegration of cell membrane, mutation of DNA and damage to membrane protein, which subsequently contribute towards propagation and development of several disease conditions such as DM, Cancer, cardiovascular disease, liver cirrhosis etc. Though the body has its own defense mechanism, but continuous exposure to xenobiotics, contaminants and chemicals causing generation of ROS drives the need for potent antioxidants to arrest irreversible damage caused due to free radicals.7 Therefore, medicinal plants, natural food and secondary metabolites present therein are the potential candidates to take care of human ailments. These medicinal plants act as rich sources of active secondary metabolites which have different structures', different mode of action and therapeutic properties as well.8 However, due to considerable health hazards associated with synthetic free radical scavengers like propyl gallate (PG), butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) their use demands a stringent regulation. Antioxidants proved to be effective in minimizing the risk of DM, enhances glucose clearance and minimizing or eliminating the complications associated with onset of DM.9 Presently used lines of treatment in the management of DM are thiazolidinediones, sulfonylureas, α -glucosidase inhibitors and buguanides are found to act by various mechanisms but with some limitations (ex. diminished therapeutic efficacy, developing resistance, etc.) This derives the researchers to focus on and explore natural resources for secondary metabolites or chemical components with valid scientific base for safe, efficacious, economic alternate in the treatment of DM and management of associated complications.¹⁰

Antigonon leptopus commonly known as Mexican creeper, coral vine, bee bush or San Miguelito vine, is a species of flowering plant in the buckwheat family, Polygonaceae. Alcohoic extract of flower showed the presence of flavonoids, phenols, saponins, phytosterols, triterpenoids, coumarins and xanthoproteins. The plant found its applications in the treatment of throat constriction, colds and low blood pressure, for pain relief and also as heart tonic. Methanolic extract of flower exerts potential antibacterial efficacy against numerous human pathogens.¹¹ Aerial parts exert significant hepatoprotective activity.¹² It is anticipated from the literature survey, that the selected plant part may reveal promising therapeutic applications in the treatment and management of DM.

MATERIALS AND METHODS

Collection and Preparation of plant extract

Flowers of *Antigonon leptopus* were collected from in and around Belagavi, Karnataka, India. Collected plant and plant parts were authenticated from Prof. R. S. Goudar, Dept. of Botany, KLES's R. L. Science Institute, Belgavi, Karnataka India. The herbarium is prepared and submitted to Department of Pharmacognosy, K.L.E.S's College of Pharmacy, Belgavi, (Karnataka) India. Extracts with presence of chemical constituents of medicinal importance were prepared by continuous hot extraction technique using ethanol followed by concentration of all extracts using rotavapor and stored 2 to 8°C. The extracts thus prepared were labeled accordingly and used for further screening.

Experimental Animals

Using Albino mice (Avg. wt. approx. 25 gm) acute toxicity study was performed. For anti-diabetic activity adult albino rats (Wistar) weighing approximately about 175 gm were selected (Ethical Clearance-Vide: JNMC/IAEC/Res-2/9/2008, date 19/12/2008) and grouped with six animals in each group for the study. Animals were fed with rat chaw and given access to water *ad libitum*. Animals were housed to ambient temperature for acclimatization with day/night cycle.

Preliminary phytochemical investigations^{13,14}

The qualitative analysis of hydro-alcoholic extract for the presence of phytoconstituents was carried out using freshly prepared reagents.

In vitro Antioxidant –DPPH free radical scavenging activity¹⁵

Antioxidant efficacy of *A. leptopus* flower extract was determined by DPPH method. Absorbance was measured in triplicate at 517 nm using Butylated hydroxytolune (BHT) as reference standard for comparison. Lower absorbance was indicative of potent free radical scavenging effect. IC_{50} value in the tested compound is, the concentration required to scavenge 50% DPPH free radical. Percentage inhibition was calculated as DPPH radical scavenging activity.

DPPH radical scavenging effect (%) = $\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control} \times 100}$

Where, Abs control is the absorbance of initial conc. of DPPH radical;

Abs sample is the absorbance of DPPH radical + sample Extract /standard

Experimental design

Pharmacological evaluation of *A. leptopus* flower extract for its free radical scavenging potential and anti-diabetic potential was performed using selected experimental animals. Alloxan was used to induce diabetes in rats. Parameters like GTT (glucose tolerance test), effect of extracts on BGL (blood glucose levels- single / multi dose treatment), Serum lipid profile study and histopathology (pancreatic) study were evaluated.

Oral glucose tolerance test (OGTT)¹⁶

Experimental animals were separated in 3 different groups with six animals in each and treatment regimen was followed as shown in Table 1. Fasting BGL (Blood

Table 1: OGTT Treatment Regimen.							
Group	Group–I (Control)	Group–II (Standard)	Group–III (Test)				
Treatment	1 ml of 1% gum acacia suspension	Glibenclamide (2.5 mg/kg-b. w.)	A. leptopus flower extract (200 mg/kg)				

Glucose Level) of each experimental animal was measured.

After ¹/₂ hr of administration of standard drug and extract as mentioned in Table 1, glucose (2 mg/kg b.w.) was administered orally. Blood was collected before and after ¹/₂, 1, 2 and 4 hr of loading glucose. For estimation of BGL; glucometer and glucostrips (glucose-oxidaseperoxidase reactive strips) were used (Wockhardt Ltd, Mumbai, India).

Effect of A. leptopus extract on blood glucose levels in alloxan induced diabetic rats

• Single dose (Acute) treatment]¹⁷

Diabetes was induced in overnight fasted rats using alloxan (120 mg/kg b.w.) single shot injection. Animals showing BGL level more than 250 mg/dl after 72 hr were selected and grouped as mentioned in Table 2 below for study.

Blood samples were collected from the tail vein and tested for BGL with help of glucometer; before and after 1/2, 1, 2, 4 and 6 hr interval after treatment as mentioned in Table 2.

Effect of A. leptopus extract on blood glucose levels and serum lipid profiles in alloxan induced diabetic rats

• Multi dose (sub acute) treatment:¹⁷

For 15 days daily once the same dose regimen as mentioned in Table 3 was given to these animals followed by collecting the blood sample on day 0, 5, 10 and 15. BGL and serum lipid profiles were measured using glucometer and autoanalyser respectively.

Histopathological studies:18

Pancreatic tissues from rats of all groups of Multi dose (Sub acute) treatment were subjected to histopathological studies using hematoxylin and eosin (H and E) stains.

Statistical analysis

Values are presented as mean \pm S.E.M. Statistical difference between treatments and the controls were tested by

Table 2: Single dose (Acute) treatment].						
Group	Group–IV (Diabetic Test)					
Treatment	1 ml of 1% gum acacia suspension	1 ml of 1% gum acacia suspension	Glibenclamide (2.5 mg/kg)	A. leptopus flower extract (200 mg/kg)		

Table 3: Multi dose (sub acute) treatment.							
Group	Group–I (Normal Control)	Group–III (Diabetic Test)	Group–IV (Diabetic Test)				
Treatment (15 days)	1 ml of 1% gum acacia suspension	1 ml of 1% gum acacia suspension	Glibenclamide (2.5 mg/kg)	A. leptopus flower extract (200 mg/kg)			

one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. A difference in mean values of P<0.05 was considered to be statistically significant.

RESULTS

Phytochemical Analysis

Antigonon leptopus (Polygonaceae) flower extract revealed the presence of alkaloids, flavonoids, phenols, phytosterols, carbohydrates, terpenoids and proteins.

In-vitro Antioxidant –DPPH free radical scavenging activity

Several concentrations ranging from 10-1000 μ g /ml of the ethanolic extract of *Antigonon leptopus* tested for their antioxidant activity by DPPH model. It has been observed that free radicals were scavenged by the extract in a concentration dependent manner (Figure 1). The extract showed DPPH radical scavenging activity with an IC₅₀ value of 439 μ g /ml when compared with Standard BHT (Butylated hydroxytolune) IC₅₀ value of 419 μ g /ml.

Acute toxicity study

It was observed that the test extracts did not manifest any significant abnormal signs, behavioral changes; body weight changes, mortality and morbidity even at 2000 mg/kg dose at the end of the 14 days of

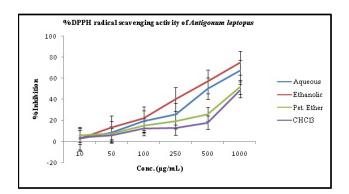


Figure 1: Free radical scavenging activity of different extracts of *Antigonon leptopus* using DPPH method.

observation. Hence, at 1/10 dose of LD_{50} pharmacological screening was performed.¹⁹

Oral glucose tolerance test (OGTT)

Ethanolicextractof *Antigononleptopus* (Flower) significantly (P<0.05) improved the glucose tolerance test up to 4 hr (Table 4). The observed reduction in blood glucose level at the end of 2nd and 4th hr was 26% and 11% respectively for *Antigonon leptopus*. The results were at par with standard drug Glibenclamide.

BGL (Blood glucose level)-Acute Study [Single dose treatment]

Administration of single dose of *Antigonon leptopus* (Flower) ethanolic extract has resulted in remarkable reduction of blood glucose level in alloxan induced diabetic rats. As Table 5, the extract exhibited significant (P<0.05) antihyperglycemic effect from 1 hr and up to 6 hr after its oral administration, compared with normal rats and diabetic control rats. After 6 hr, blood glucose lowering potential percentage of *Antigonon leptopus* was 16%, while standard drug Glibenclamide (2.5mg/kg) achieved 20% reduction when compared with diabetic control rats.

BGL-Sub Acute Study [Multi dose treatment]

The blood glucose level of each animal was monitored on 0th, 5th, 10th and 15th days after administration of *Antigonon leptopus* (Flower) ethanolic extract. As shown in the Table 6 initial anti-diabetic activity was observed on 5th day and continued to increase in all groups during the experimental period. During multi-dose treatment period, the extract caused a significant decrease of 30%, 40% and 54% in blood glucose levels on 5th, 10th and 15th day intervals, respectively, when compared with diabetic control group.

Effect of Antigonon leptopus (Flower) ethanolic extract on serum lipid profiles in alloxan induced diabetic rats [Multi dose (sub acute) treatment]

Lipid lowering potentials of ethanolic extract of *Antigonon leptopus* (Flower) in hyperlipidemic rats is given in Table 7. Treatment with ethanolic extract of

Table 4: Oral glucose tolerance test (OGTT).							
Animal Group	Treatment	Blood glucose concentration (mg/dl) (Mean ± SEM)					
(n= 6)		0 min	30 min	60 min	120 min	240 min	
I	Normal control (1% gum acacia)	92.8± 2.9	161.6± 4.1	162.7±2.6	161.2± 3.5	132.3± 3.2	
II	Glibenclamide 2.5 mg/kg)	96.0±2.4	143.0±2.4 *	145.8±1.2 *	137.0±2.2 *	98.9±1.2 *	
III	A. leptopus flower extract (200 mg/kg)	98.2±4.2	130.0±2.7 *	133.2±3.5 *	124.7±2.6 *	109.0±1.4 *	

*P < 0.01. Significant, compared to normal control

n = no of animals in each group

	Table 5: BGL (Blood glucose level)-Acute Study.								
Animal Group	Treatment	Blood glucose concentration (mg/dl) (mean ± S.E.M)							
(n= 6)	mouthout	0 min	30 min	60 min	120 min	240 min	360 min		
I	Normal control (1% gum acacia)	87.2±2.4	88.4±2.1##	90.50±2.3 ##	90.86±1.8 ##	94.4± 2.2 ##	95.76± 3.1##		
11	Diabetic control	288.8±5.0	296.4±5.1*	297.1±5.2*	291.3±4.1*	293.5±3.5*	294.0± 4.1*		
111	Glibenclamide (2.5 mg/kg)	298.3±6.2	285.1±7.0*	271.3±9.3*	259.0± 11.9* #	253.8± 12.1*##	236.9 ± 11.8*##		
IV	Et. AL (200 mg/kg)	278.1± 5.0	273.4± 5.5*#	265.2 ± 6.0*##	254.8±6.2*	248.1± 6.6*##	234.7 ± 6.3*##		

*P < 0.01. Significant, compared to normal control,

*P < 0.05 and **P < 0.01 Significant, compared to diabetic control

n = no of animals in each group

Table 6: BGL-Sub Acute Study.								
Animal Group (n= 6)	Treatment	Fasting blood glucose concentration (mg/dl) (mean ± S.E.M)						
		0 th Day	5 th Day	10 th Day	15 th Day			
I	Normal control (1% gum acacia)	87.35±2.2	87.75±2.8##	87.35±2.3##	88.35±1.8 ^{##}			
II	Diabetic control (1% gum acacia)	288.2±5.2	277.6±7.1**	268.3±4.2**	256.8±4.1**			
III	Glibenclamide (2.5 mg/kg)	300.3±6.9	222.3±12.3** ##	190.3±12.8**##	172.3±14.3** ##			
IV	Et.AL (200 mg/kg)	278.2±8.7	194.2 ±10.2** ##	166.4±9.6** ##	122.8±8.4*##			

*P < 0.05 and **P < 0.01 Significant, compared to normal,

n = no of animals in each group

*P < 0.05 and **P < 0.01 Significant, compared to diabetic control

Table 7: Serum profile in diabetic rats after 15 days of treatment.								
Exp. Group (n= 6)	Treatment	TGL mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl	Total Cholesterol mg/dl		
I	Normal control (1% gum acacia)	79±3.01	44.7±3.7	26±1.9	21.2±2.2	53.3±2.5		
II	Diabetic control	120±3.9	38.3±0.9	49.7±3.5	80.5±4.2	88.2±2.9		
III	Glibenclamide (2.5 mg/kg)	112±4.6*	59.2±1.9**#	53.3±3.8*	45.8±4.0**	75.5±2.2		
IV	Et. AL (200 mg/kg)	90.7±2.7**	42.5±1.7*	22.5±1.2**	32.7±1.7**	60.7±1.5**##		

*P< 0.05 and **P< 0.01 Significant, compared to normal,

n = no of animals in each group

*P< 0.05 and **P< 0.01 Significant, compared to diabetic control

Antigonon *leptopus* (Flower) (200 mg/kg) and Glibenclamide in hyperlipidemic rats for 15 consecutive days, resulted in significant decrease in serum total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and Very low-density lipoprotein (VLDL) while there was a marked increase in high density lipoprotein (HDL).

Histopathological studies

Histopathological examination of pancreas of experimental animals showed substantial regeneration of Islets of Langerhans and β cells after treating with ethanolic extracts of *Antigonon leptopus* (Flower) and

Glibenclamide, which were earlier, under necroses by alloxan. Figure 2 (A-D) depicts the islets of pancreas in different groups. Photomicrographs (A) of normal healthy control group showed normal acini and normal cellular population of islets of Langerhans. However, in alloxan treated rats, there was extensive damage of islets of Langerhans and they appeared to be irregular (B). Treatment of diabetic rats with glibenclamide showed moderate expansion of cellular population and size of islet cells (C). However, extract (200 mg/kg) treateddiabetic rats showed partial restoration of normal cellular population and size of islet cells (D).

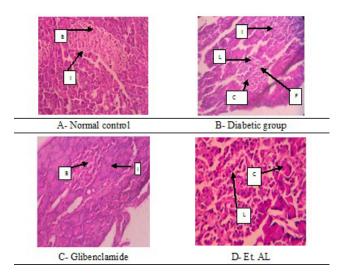


Figure 2: Histopathology of Pancreas.

DISCUSSION

Diabetes mellitus was found to be an outcome of metabolic disorder of fats, carbohydrates and proteins. This may be attributed to deficiency (absolute or partial) of insulin as well as developed insulin resistance. DM is characterized by elevated glucose levels in blood which in turn results in various complications associated with morbidity and mortality. The associated complications such are kidney damage, blindness, cardiac disease, stroke, end organ damage and amputations.²⁰ Though there is a substantial development in the management and treatment of DM, synthetic anti-diabetic medicines (biguanides, thiazolidinediones, sulphonylureas or insulin) faces many limitations. They have major adverse effects and found to be inefficient in managing the diabetic complications effectively. The information about multifarious or diverse state of this disease condition drives us to search for more promising drug candidates with devoid of side effects.²¹ However, Indian medicinal plants are reported to be highly useful in the effective management of DM due to its abundance and less or no side effects.²² Herbal drugs are believed to act by restoration of pancreatic function either by increased insulin secretion or by arresting intestinal glucose uptake. Therefore, it was observed that, herbal drugs act by protecting β -cells and smoothens variations in blood glucose levels. It was suggestive that, the secondary metabolites present in plants, such as alkaloids, flavonoids, glycosides and terpenoids etc are contributing factors for anti-diabetic efficacy.23 Further; abridged plasma antioxidant efficacy, adds to oxidative stress, which is a major factor in development of DM. These produced reactive oxygen species (ROS) results in tissue damage and gradual increase in β -cells dysfunction.

Herbal drugs are believed to act by various mechanisms such as inhibition of glucose absorption, diminished enzyme activities responsible for blood glucose synthesis, increasing insulin sensitivity and production, also, quantum of soluble fibers present in plants contributes significantly in reducing glucose level. In addition free radical scavenging activity of plants has remarkable beneficiary effect in management of DM.24 Plants are rich sources of secondary metabolites possessing antioxidant activity, such as flavonoids, Vitamin C, phenols, terpenoids, carotenoids, anthraquinones, steroids, strychnine and alkaloids. For determination of antioxidant potential DPPH method was used. From the results it is evident that ethanolic extract of Antigonon leptopus is having potential free radical scavenging activity. This activity is attributed due to the presence of phenols, flavonoids terpenoids and such other chemical compounds present. Further, a promising antihyperglycemic effect of ethanolic extract of Antigonon leptopus was observed by improvement in oral glucose tolerance test, decreased blood glucose levels with a single dose (acute study) and multi dose (Sub acute) studies in experimental animals (Alloxan induced diabetic rats). Ethanolic extract of Antigonon leptopus and standard drug Glibenclamide showed considerable efficacy in lowering the blood glucose level in oral glucose tolerance test. This supports the hypothesis that protection and regeneration of β cells is the major contributing factor for anti-diabetic activity. Also, it may act by exerting inhibitory effect on hormones responsible for elevated blood glucose, improvement in affinity and sensitivity of insulin, diminished glycogen production, optimum utilization of glucose by the body, improved circulation in the body and correcting the lipid and protein metabolism.²⁵ In present study, damaged pancreas was observed in alloxan treated (diabetic) experimental animals (Figure 2B). Protection and regeneration of β cells was reported in glibenclamide-treated group (Figure 2C). Considerable similar results as of glibenclamide-treated group were observed with ethanolic extract treated group (Figure 2D). It was suggestive by photomicrographs that healing of damaged pancreas may be a valid reason and probable mechanism for its anti-diabetic potency of ethanolic extract of Antigonon leptopus. The anti-diabetic efficacy of Antigonon leptopus may be due to the secondary metabolites-flavonoids, phenols and terpenoids present in it. Numerous medicinal plants extracts and few of chemical constituents (flavonoids, tannins and quinones) are widely used for the treatment and management of DM.²⁶ It was observed that, the experimental animals treated with ethanolic extract of Antigonon leptopus

showed a significant decrease in levels of blood glucose, total cholesterol, triglycerides, LDL and VLDL whereas increase in HDL levels were recorded. From the results obtained and inferred, it validates the claims that *Antigonon leptopus* could play a major role in scavenging the free radicals effectively and lowering blood glucose levels and maintain the lipid ratio so as to exert a promising anti-diabetic as well as hypolipidemic activity. Also, unlike other synthetic anti-diabetic and lipid lowering drugs, *Antigonon leptopus* is devoid of such adverse effects.

CONCLUSION

It is evident from the study results that antioxidant activity, protection and regeneration of β cells, increased insulin sensitivity and optimum utilization of glucose by the body are the plausible mechanism for anti-diabetic activity of ethanolic extract of *Antigonon leptopus*. Moreover, the lipid lowering activity observed reveals promising alternative for treatment of hyperlipidemic patients with less or no side effects. It can be concluded that *Antigonon leptopus* can be used as a valuable alternative from natural resource as a hypoglycemic and hypolipidemic agent. However, further, fractionation and isolation of lead compounds from the extract, responsible for observed therapeutic efficacy; should be carried out.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Abbreviations Used

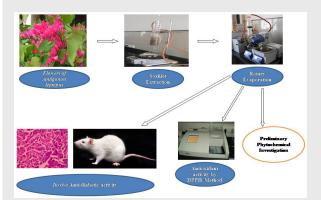
DM: Diabetes mellitus; **DPPH:** 1,1-diphenyl-2picrylhydryzyl; **BGL:** Blood glucose level; **ROS:** Reactive oxygen species; **Et. AL:** Ethanolic extract of *Antigonon leptopus*; **OGTT:** Oral glucose tolerance test.

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PICTORIAL ABSTRACT



SUMMARY

Current study; deals with extraction and evaluation of ethanolic extracts of flowers of Antigonon leptopus for its antioxidant and anti-diabetic efficacy using DPPH and animal models respectively. Phytochemical analysis revealed presence of alkaloids, flavonoids, phenols, phytosterols, carbohydrates, terpenoids and proteins. It was observed that the extract exhibited promising free radical scavenging effect. Further, anti-diabetic screening showed significant reduction in blood glucose level in acute and sub acute study. Also, remarkable lipid lowering potential was recorded with ethanolic extract of Antogonon leptopus. It can be summarized and stated that, antioxidant efficacy, anti-diabetic action and lipid lowering property are complementary to each other for effective management of diabetes mellitus.

About Authors



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