ISSN 0975 – 9328

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

ANTIDIABETIC ACTIVITY OF AERIAL PARTS OF *ANTIGONON LEPTOPUS* HOOK. & ARN. IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

To evaluate the antidiabetic activity of methanolic extract of aerial parts of *Antigonon leptopus* Hook & Arn. (MEAL) in alloxan induced diabetic rats. A single dose of alloxan monohydrate (120mg/kg, i.p.) was used to induce diabetes mellitus. Diabetes was confirmed by the elevated blood glucose levels determined at 72h. Animals with fasting blood glucose (FBG) level more than 250mg/dl were considered as diabetes. The MEAL at the dose of 200 and 400mg/kg and the standard drug glibenclamide (2.5mg/kg) were administered orally to the diabetic rats for 15 days. The FBG levels were estimated on 0, 5, 10 and 15th day. On 15th day, the blood was collected and serum is separated for the estimation of biochemical parameters and the pancreas was isolated for histopathological studies. The MEAL at 200 and 400mg/kg produced a dose-dependent fall in fasting blood glucose levels. The results of biochemical parameters and histopathological studies suggest that, MEAL possesses significant antidiabetic property. The methanolic extract of aerial parts of *Antigonon leptopus* Hook & Arn. possess significant antidiabetic activity. This study supports the traditional claim and the methanolic extract of this plant could be added in traditional preparations for the ailment of various diabetes-associated complications.

Keywords: Diabetes mellitus, *Antigonon leptopus* Hook & Arn., Antidiabetic activity, Alloxan induced diabetes, Glibenclamide.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels and disturbances in carbohydrates, fats and protein metabolism. These metabolic abnormalities resulting from defects in insulin secretion and/or insulin action (Fajans *et al*., 1997; George and Rudvid, 2000; Nyholm *et al*., 2000). Diabetes mellitus is affecting approximately 5% of the world population (King *et al*., 1998). The prevalence is predicted to increase alongside world figure which is estimated to hit 220 millions by the year 2010 (Amos *et al*., 1997). The countries with the largest number of diabetic people in the year 2025 will be India, China, United States (Ramachandran *et al*., 2002). The chronic hyperglycemia of diabetes is associated with damage, dysfunction and failure of various organs over long term (Lyra *et al*., 2006). Due to adverse reactions and other undesirable side effects of present synthetic drugs, there is continuous search for alternative drugs. Therefore it is prudent to look for option in herbal medicines for the management of diabetes. Although, herbal medicines have long been used effectively for treating diseases in Asian communities and throughout the world (Bailey and Day, 1989).

Antigonon leptopus Hook & Arn. (Family: Polygonaceae) or coral vine is native Mexico and commonly found in tropical Asia, Africa, the Caribbean

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and the Americas (Raju *et al*, 2001). It is commonly grown in gardens and often run wild. It is a climbing vine; stems slender. Leaves are alternate, cordate-ovate or triangular, entire, acute to acuminate. Flowers are bright pink, in panicled racemes that terminate in tendril. Fruits of 1-seeded, hard nut let, 3-gonous, biconvex, compressed (Madhava Chetty *et al*., 2008). Traditionally, *Antigonon leptopus* Hook. & Arn. have been used to treat diabetes, asthma, liver and spleen disorders, cough and throat constriction (Cheryl A Lans, 2006; Idu and Onyibe, 2007; Mitchell and Ahmad, 2006). Therefore we attempt to investigate the antidiabetic activity of methanolic extract of aerial parts of *Antigonon leptopus* Hook. & Arn. (MEAL) in alloxan induced diabetic rats to support the claim.

MATERIALS AND METHODS

Plant collection

The aerial parts of *Antigonon leptopus* Hook & Arn. was collected from Tirupati, Andhra Pradesh, in the month of August 2009. The plant was authenticated by Prof. P. Jayaraman, Director of National Institute of Herbal Science, West Tambaram, Chennai. The voucher specimen (PARC/2009/350) of the plant was deposited at the college, for further reference.

Preparation of plant extract

The aerial parts of *Antigonon leptopus* Hook & Arn. were dried in shade and pulverized in grinder-mixer to obtain a coarse powder. It was then passed through the 40 mesh sieve. A weighed quantity (210gm) of the powder was subjected to continuous hot extraction with methanol in Soxhlet apparatus for 48h. The extraction was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. The percentage of yield of methanolic extract of *Antigonon leptopus* Hook & Arn. was found to be 32.91%w/w.

Animals used

Male Wistar albino rats (150-200g) were obtained from the animal house in Sreevidyanikethan College of Pharmacy, Tirupati, Andhra Pradesh. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref. No. IAEC / XIII / 05 / SVCP / 2008-2009).

Acute toxicity study

The acute toxicity methanolic extracts of *Antigonon leptopus* Hook & Arn. was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not produced mortality and morbidity even at 2000mg/kg dose. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study (OECD, 2002).

Induction of diabetes mellitus

Diabetes Mellitus was induced in overnight fasted adult male Wistar albino rats weighing 150-200 g by a single intraperitoneal injection of 120 mg/kg alloxan monohydrate. Hyperglycemia was confirmed by the elevated glucose levels determined at 72h. This model has been used in earlier studies to induce diabetes in rats (Venkatesh *et al.*, 2008, Neeli *et al.*, 2007). Animals with blood glucose level more than 250 mg/dl were considered as diabetic. Rats found with permanent Diabetes were used for the antidibetic study.

Experimental design

Animals were divided into five groups, each consisting of six rats. The extracts were administered for 15 days. Group-I: Normal control rats administered normal saline (0.9% w/v); Group-II: Diabetic control rats administered normal saline (0.9% w/v); Group-III: Diabetic rats administered standard drug glibenclamide (2.5 mg/kg); Group-IV: Diabetic rats administered MEAL (200 mg/kg); Group-V: Diabetic rats administered MEAL (400 mg/kg) daily for 15 days.

Antidiabetic study

The effects of administration of MEAL extracts in diabetic rats were observed by measuring fasting blood glucose levels and serum lipid profile. Fasting blood glucose was estimated on days 0, 5, 10 and 15 of extracts administration. The biochemical parameters (TG, TC, HDL, LDL, and VLDL) and the histopathological studies of the pancreas were determined on day 15 after the animals were sacrificed by decapitation.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Tukey-Kramer multiple comparison test, the p values less than 0.05 were considered as significance.

RESULTS

Acute toxicity study

Acute toxicity study in which the animals treated with the MEAL at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Effect of MEAL on fasting blood glucose levels of alloxan induced diabetic rats

The MEAL at the dose of 200 and 400mg/kg, p.o. produces a dose-dependent fall in fasting blood glucose level (FBG). After 15 days of treatment, the maximum reduction in FBG was observed in the treated group rats (Groups- IV and V) as compared with untreated group (Group-II). On the progression of treatment with MEAL, FBG reduced from 5th day. At the end of experiment (15th day) FBG levels was 127.16 ± 4.41 and 120.5 ± 1.28mg/dl in the doses of MEAL 200 and 400mg/kg respectively. The changes in FBG of all groups were represented in Table-1.

Effect of MEAL on lipid profile in alloxan induced diabetic rats

The levels of serum lipid profile such as triglycerides (TG), total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), and very low density lipoproteins (VLDL) of the all groups are shown in Table-2. The levels of TG, TC, LDL and VLDL were significantly higher as well as levels of HDL significantly lower in diabetic control rats as compared to normal rats. The MEAL at the dose of 200 and 400mg/kg were significantly decreases in the levels TG, TC, LDL, and VLDL as well as increases in the level HDL as compared to diabetic control rats.

Effects MEAL on pancreas histopathological changes

Architecturally, pancreatic islet of diabetic control rats (group-II) shown significantly destruction as compared to normal control animals (group-I). Pancreatic islets of rat which are treated with MEAL 200 and 400mg/kg (groups-IV and V) also showed architectural destruction but to a lesser extent as compared to diabetic control rats. The architectural of pancreatic islets of animals treated with standard drug glibenclamide showed similar to normal animal group (Fig. 1-5).

Table 1. Effect of MEAL on fasting blood glucose levels of alloxan induced diabetic rats

Groups (n=6)	Fasting Blood Glucose Levels (mg/dl)			
	0th Day	5th Day	10th Day	15th Day
Group-I (Normal Control)	96 ± 1.15***	95.66 ± 1.14***	95.16 ± 0.79***	95.5 ± 0.76***
Group-II (Diabetic Control)	289.5 ± 4.47	292.33 ± 4.19	292.33 ± 4.04	293.66 ± 3.96
Group-III (Glibenclamide-2.5mg/kg)	271.5 ± 5.05	216.33 ± 3.24***	141.16 ±5.56***	100 ± 0.66***
Group-IV (MEAL-200mg/kg)	271.5 ± 6.75	241.5 ± 10.97***	173.66 ± 9.46***	127.16 ±4.41***
Group-V (MEAL-400 mg/kg)	288.16 ± 7.89	239 ± 7.44***	181.83±19.04***	120.5 ± 1.28***

Values are expressed as mean ± SEM of 6 rats in each group. *** p<0.001, Group III,IV &V compared to Group II (diabetic control group).

Table 2. Effect of MEAL on lipid profile in alloxan induced diabetic rats

Groups (n=6)	Biochemical Parameters				
	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group-I (Normal Control)	81.72±0.43***	80.86±0.17***	32.39±0.50***	32.54±0.63***	16.01±0.90***
Group-II (Diabetic Control)	133.57 ± 1.10	142.72 ± 5.89	16.87 ± 4.19	76.57 ± 4.19	49.2 ± 5.12
Group-III (Glibenclamide-2.5mg/kg)	83.14±0.61***	83.00±0.49***	29.61±0.34***	32.47±0.25***	20.92±0.58***
Group-IV (MEAL-200mg/kg)	102.45±0.59***	93.58±0.44***	24.39±0.25***	39.65±0.59***	29.52±0.44***
Group-V (MEAL-400 mg/kg)	91.00 ± 0.47***	88.04±0.49***	26.93±0.39***	36.07±0.35***	25.07±0.47***

Values are expressed as mean ± SEM of 6 rats in each group. *** $p<0.001$, Group III,IV &V compared to Group II (diabetic control group). TG = Triglycerides, TC = Total cholesterol, HDL = High density lipoproteins, LDL = Low density lipoproteins,, VLDL = Very low density lipoproteins,.

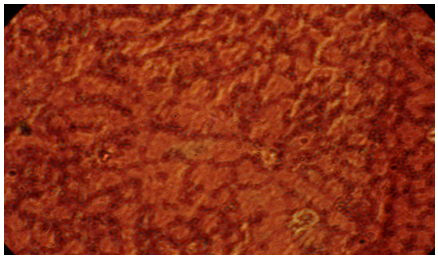

Fig.1. Normal control

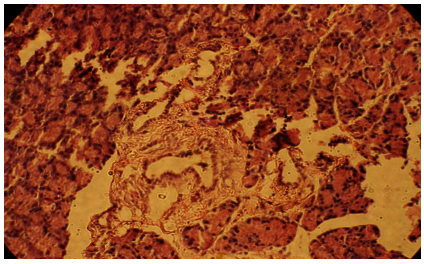

Fig.2. Diabetic control

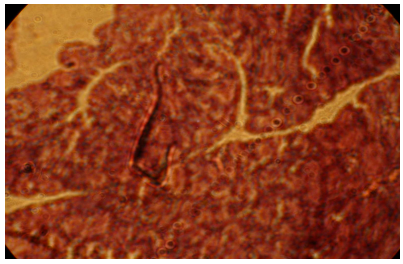

Fig.3. MEAL-200mg/kg

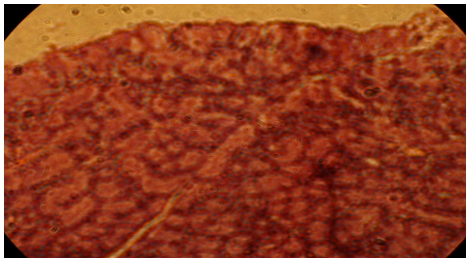

Fig.4. MEAL-400mg/kg

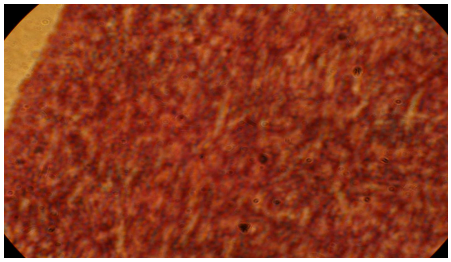

Fig.5.Glibenclamide-2.5mg/kg

DISCUSSION AND CONCLUSION

Diabetes mellitus is a complex metabolic disorder, causes the hyperglycemia and severe alterations of glucose and lipid metabolism. This metabolic abnormalities leads to an increased generation of reactive oxygen species (Rajasekaran *et al*., 2006). Alloxan has been observed to cause a massive reduction in the number of the β-cells of the islets of langerhans and induce hyper glycemia (Goldner and Gomori, 1943). The diabetogenic action of alloxan is mediated by reactive oxygen speciese, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of pancreatic β-cells which reduces the synthesis and the release of insulin (Szkudelski, 2001; Sakurai *et al.*, 2001). It is well documented that, decreased anti- oxidant enzymes leaves and enhanced lipid peroxidation observed in alloxan-induced diabetes. (Roy *et al*., 2005; Sepici-Dincel *et al*., 2007).

In this study effect of MEAL on hyperglycemia is evaluated in alloxan-induced diabetic rats. It was found that the fasting blood glucose levels (FBG) of the animals which are treated with MEAL (Groups IV & V) and the standard drug glibenclamide (Group-III), significantly reduces when compared with diabetic control (untreated) group. The FBG of all groups were observed on 0^{th}, 5^{th}, 10^{th}, and 15^{th} day. The diabetic rats which treated with MEAL and glibenclamide showed a significant decrease in blood glucose level on 5^{th}, 10^{th} and 15^{th} day. On 15^{th} day FBG of Group-III decreases nearly too normal range. In the case of Group-IV & V animals FBG significantly reduces, but it was some less when compared to Group-III. When compared with untreated group, the MEAL at the dose of 200 & 400 mg/kg significantly reduces the hyperglycemia. These results give us the suggestion that MEAL having significant hypoglycemic effect.

Lipids play an important role in the pathogenesis of diabetes. The levels of serum lipids are usually increased in diabetes mellitus and such elevation contribute to coronary heart disease (Al-Shamaony et al., 1994). It is well documented that untreated diabetes mellitus, there will be increase in total cholesterol (TC), triglycerides (TG), VLDL and LDL cholesterol associated with decrease in HDL cholesterol (Arvind *et al.*, 2002). In the present investigation the TG, TC, LDL and VLDL cholesterol was increased in diabetic control animals (untreated group) and it was decreased in 15 days treatment with MEAL as well as HDL cholesterol level was significantly increased. The total lipid profile in serum (TG, TC, HDL, LDL, VLDL) of the alloxan induced diabetes animals treated with MEAL (200 and 400 mg/kg) was substantially improved, as compared to diabetic control group. These results give us the suggestion that MEAL may inhibit the cholesterol synthesis pathway and increased HDL/LDL ratio may be due to the activation of LDL receptors in hepatocyte, which is responsible for taken up LDL into the liver and reduce the serum LDL level (Rand *et al*., 1999).

It is well documented that the phytoconstituents comes under the category of alkaloids, triterpenoids, aminoacids, steroids, flavonoids, phenolics, coumarins, iridoids, polysaccharides, glycopeptides and guanidines are reported to have antidiabetic activity (Grover *et al*., 2002; Pulok *et al*., 2006). The following chemical constituents are specifically reported to have antidiabetic activity; quercetin, beta-sitosterol, kaempferol glucosides, ferulic acid etc. (Li *et al*., 2004; Pulok *et al*., 2006; Mohamed *et al*., 2006; Satyavati *et al*., 1989). The preliminary phytochemical screening of the methanolic extract of *Antigonon leptopus* Hook. & Arn. revealed that the presence of steroids, flavonoids, tannis, alkaloids and glycosides (William Carey Mamidipalli *et al*., 2008). The functional food components of *Antigonon leptopus* have been reported by Mulabagal vanisree *et al*. Purification of the methanolic extract yielded; n-hentriacontane, ferulic acid, 4-hydroxycinnamic acid, quercetin-3-rhamnoside and kaempherol-3-glucoside; along with beta-sitosterol, beta-sitosterol-glucoside and d-manitol (Mulabagal vanisree *et al*., 2008). The antidiabetic activity of *Antigonon leptopus* Hook. & Arn. may be attributed due to presence of these constituents. This study supports the traditional claims and the methanolic extract of this plant could be added in traditional preparations for the ailment of various diabetes associated complications.

It is concluded from the data, that the methanolic extract of aerial parts of *Antigonon leptopus* Hook. & Arn. possesses significant antidiabetic activity and may prove to be effective for the treatment of diabetes mellitus. However, longer duration studies on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent antidiabetic drug.

Acknowledgement

The authors wish to our beloved Chairman. Padmasree Dr. M. Mohan Babu, for his generous support for this study. This research was supported by the grants from Sree Vidyanikethan College of pharmacy.

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