ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Antidiabetic activity of *Anisomels indica* Kuntze leaf flavonoid fraction in normal and alloxan induced diabetic mice

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Manuscript Info

Manuscript History:

Received: 25 September 2015
Final Accepted: 26 October 2015
Published Online: November 2015

Key words:

Antidiabetic, *Anisomeles indica*, Alloxan, Blood glucose level, Hypoglycemic.

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Abstract

Diabetes mellitus is a major health problem in most of the countries. Purpose of the study was to evaluate the antidiabetic activity of leaf flavonoid fraction (LFF) of *Anisomeles indica* in normal and alloxan induced diabetic mice. Alloxan monohydrate 150 mg/kg body weight was used to induce diabetes mellitus. The LFF of *A. indica* at 500 mg and 1000 mg and glibenclamide at 7 mg/kg body weight administered individually to normal and alloxan induced diabetic mice significantly reduced the blood glucose. Also the administration of LFF significantly decreased serum total cholesterol, triglyceride and aspartate transaminase and alanine transaminase levels. Oral glucose tolerance test was performed by administration of *A. indica* LFF at 500 and 1000 mg/kg body weight and glibenclamide at 7 mg/kg body weight to different groups of animals. *A. indica* LFF and glibenclamide significantly lowered blood glucose levels at all time points that were sampled after oral glucose load. Results of the present study suggest that, *A. indica* LFF possess antidiabetic property and effective in lowering blood glucose level in diabetic mice.

INTRODUCTION

Diabetes mellitus (DM) is a syndrome initially characterized by loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting impaired metabolism of not only glucose but also other energy yielding fuels such as lipids and proteins (Scheen, 1990). Diabetes mellitus is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin (Matsui et al., 2007). Diabetes mellitus is currently one of the most costly and burdensome chronic diseases and is a condition that is increasing in epidemic proportions throughout the world. Diabetes is a serious illness with multiple complications and premature mortality, accounting for at least 10% of total health care expenditure in many countries (King et al., 1998). The prevalence of diabetes of all age groups worldwide is projected to rise from 171 million in 2000 to 366 million in 2030 (Amos et al., 2010). Reason for this rise includes increase in sedentary lifestyles, consumption of energy rich diet, obesity higher life span, etc. (Yajnik, 2001).

Several approaches are presently available to reduce the hyperglycemica including insulin therapy, treatment by sulphonylureas. Unfortunately, all of these therapies have limited efficacy and various side effects, and thus searching for new classes of compounds is essential to overcome these problems.

Based on the WHO recommendations hypoglycemic agents of plant origin are used in traditional medicine (WHO, 1980). The attributed antihyperglycemic effects of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose. Hence treatment with herbal drugs has an effect on protecting β-cells and smoothing out fluctuation in glucose levels.

Most of these plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc. that are frequently implicated as having antidiabetic effects (Loew and Karzkin, 2002).

Anisomeles indica (L) Kuntze belongs to the family of *Labiatae*, in India commonly called as Indian cat mint and kalabhangra. Published reports on *A. indica* substantiate that, aqueous extract were used in ethnobotany to treat disorders like inflammation, gastric dysfunction (Arisawa, 1986; Dharmasiri, 2003). *A. indica* has been shown to contain a variety of secondary metabolites including flavonoids, diterpenoids, phenylpropanoids, steroids, essential oil (Manchand and Blount, 1977; Ansari and Dobhal, 1982; Rao *et al.*, 1983 a,b,c, 1984, 1985; Arisawa et al., 1986 a,b; Dhobhal et al., 1988; Shahidul Alam et al., 2000).

In the present study leaf flavonoid fraction (LFF) of *A. indica* was evaluated for the antidiabetic activity in normal and alloxan induced diabetic mice.

MATERIALS AND METHODS

Plant material

A. inidca Kuntze plants at the pre-flowering stage (between September to November) were collected from a field area of Davangere University campus and authenticated by Prof. Pushpalatha, Departmen of Botany, Sahyadri Science College, Kuvempu University, Shivamogga, India. Leaves were clipped, shade dried and pulverized in a mechanical grinder. The coarse powder was passed through sieve No. 40 and used for further studies.

Preparation of leaf flavonoid fraction

Leaf powder was defatted with petroleum ether at room temperature for about 24 hours. Extract was filtered-off and shade dried. Dried material was re-extracted with methanol and concentrated under reduced pressure at 30 ºC. Combined concentrates were purified by subjecting to partition against ethyl acetate to remove chlorophylls, stilbenoids, less polar flavonoids and other non-polar compounds from the mixture. The non-aromatic water soluble compounds other than flavonoids like free sugars, aliphatic acids present in the extract obtained after the liquid-liquid partition step were removed by subjecting extract to adsorption chromatography using amberlite XAD-4, which specifically absorbs aromatic compounds including flavonoids, anthocyanins, whereas free sugars and non-aromatic compounds were removed by washing with distilled water, until eluted water has a neutral pH. The adsorbed crude flavonoids were eluted using methanol (Markhalm, 1982). Obtained flavonoid fraction concentrated under reduced pressure and powder was used for administration to animals in the experiment.

Quantitative estimation of leaf flavonoid fraction

Estimation of total phenolic content

Total phenolic content in leaf flavonoid fraction was quantified using Folin-ciocalteu reagent (Chang et al., 2002). Briefly, 1 ml of LFF (50) was mixed with Folin–Ciocalteu reagent (diluted 1:10 v/v) followed by the addition of 2 ml of sodium carbonate (7.5%, w/v) and mixed, allowed to stand for 90 min at room temperature and absorbance was measured at 750 nm using spectrophotometer (Systronics doublebeam spectrophotometer 2202). A calibration curve was generated using the gallic acid standard. Total phenolic content was expressed as gallic acid equivalent (GAE)-TPC mg/100 g.

Estimation of total flavonoid content

Total flavonoid content of LFF was determined according to the modified method of Zhishen et al. (1999). Briefly, 1 ml of LFF was mixed with 4 ml distilled water. To this 0.3 ml of 5% sodium nitrite was added, after 5 min 0.3 ml of 10% aluminum chloride was added. After 1 min 2 ml of 1 M sodium hydroxide was added and the total volume in test tube was made up to 10 ml by adding 2.4 ml of millipore water. The reaction mixture was mixed well and absorbance was measured at 510 nm. A calibration curve was generated by using the catechin standard. The Total flavonoid content was expressed as catechin equivalents (TFC mg/100 g).

Animals

Healthy Swiss albino mice weighing 25-30 g were used for the study. Animals were housed in poly propylene cages under condition of temperature (27±2˚C) with a 12 h light-dark cycles. Animals were acclimatized for 10 days before the study. They were fed with commercial rodent diet (VRK laboratory animal feeds, Bangalore) and water *ad libitum*.

Oral Glucose Tolerance Test

Oral glucose tolerance test (Bonner-weir, 1988) was performed in overnight fasted (18 h) normal mice. The mice were categorized into 4 treatment groups of six mice in each. Group 1 served as normal control administered orally phosphate buffer saline (PBS) (pH 7.4), group 2 administered orally standard drug glibenclamide at a dose of 7 mg/kg b.w., group 3 and 4 administered orally 500 and 1000 mg/kg b.w., of *A. indica* LFF dissolved in PBS

respectively. Soon after 30 min of administration of drugs animals in all the groups were orally loaded with 2 g/kg b.w., of glucose. Blood glucose estimations were done just before the glucose administration and at 30, 60, 120 and 150 min after the glucose administration, using glucometer (One touch, blood glucose monitoring system, Lifescan, Europe) and strips which works on the principle of glucose oxidase method.

Hypoglycemic activity in normal mice

Healthy Swiss albino mice weighing 25-30 g were fasted overnight and were divided into 4 groups of 6 mice each.
Group 1: Normal mice received orally PBS
Group 2: Normal mice received orally standard drug glibenclamide (7 mg/kg b.w.)
Group 3: Normal mice received orally LFF of *A. indica* (500 mg/kg b.w.) dissolved in PBS
Group 4: Normal mice received orally LFF of *A. indica* (1000 mg/kg b.w.) dissolved in PBS
Blood samples were collected from tail before and after 1, 2 and 4 h of treatment, and the glucose levels were determined using glucometer.

Induction of Diabetes

For induction of diabetes in Swiss albino mice, 150 mg/kg b.w. of alloxan monohydrate dissolved in PBS was administered intraperitoneally to overnight fasted mice (Anitha et al., 2005). After 1 h the animals were fed with standard pellet and water *ad libitum*. After 72 h, the blood glucose levels were estimated and mice having blood glucose levels more than 180 mg/dl were selected for the study.

Hypoglycemic activity in diabetic mice

Healthy Swiss albino mice weighing 25-30 g were fasted overnight and were divided into 5 groups of 6 mice each.
Group 1: Normal control received orally PBS.
Group 2: diabetic mice received orally PBS.
Group 3: Diabetic mice treated orally with standard drug glibenclamide (7 mg/kg b.w.) daily for 28 days.
Group 4: Diabetic mice treated orally with LFF of *A. indica* (500 mg/kg b.w.) dissolved in PBS.
Group 5: Diabetic mice treated orally with CFF of *A. indica* (1000 mg/kg b.w.) dissolved in PBS.

Blood samples were collected before and after I, II, III and IV weeks of treatment, and glucose levels were determined using glucometer.

Biochemical analysis

At the end of the experiment animals were fasted overnight, sacrificed by cervical dislocation, blood samples were collected, allowed to clot and serum separated by centrifugation at 3000 rpm for 10 min. Serum glucose, total cholesterol, triglycerides, aspartate transaminase (AST), alanine transaminase (ALT) were determined by using autoanalyzer and commercial kits (Prie test, Touch, Semi-autobiochemistry analyzer, Robonik, India).

Statistical analysis

Statistical analysis was performed using one-way analysis of (ANOVA), followed by student's t-test. The values represented are mean±SD for six mice in each group. Statistical significance was considered at $p<0.05$.

RESULTS

Total phenols and flavonoid content were determined in the LFF and data presented in Table 1.

Effect of LFF in Oral Glucose Tolerance Test in normal mice

Blood glucose levels were determined in the control, glibenclamide and *A. indica* LFF treated animals at different time points (0, 30, 60, 120,150 min) after oral administration of glucose (2 g/kg). There was a peak increase in the blood glucose levels at 30 min in all the groups. In glibenclamide and *A. indica* LFF 1000 mg/kg b.w. treated groups, there was a decrease in blood glucose level at 150 min compared to control group (Table 2).

Effect of LFF on blood glucose level in normal fasted (euglycemic) mice

Administration of glibenclamide and *A. indica* LFF (500 and 1000 mg/kg b.w.) on euglycemic mice was not significant at 1 h, while it was significant at 4 h ($p<0.05$) as compared to control group mice(Table 3).

Effect of CFF on blood glucose level in alloxan induced diabetic mice

The levels of blood glucose in normal and diabetic mice at 0, 1, 2, 3 and 4 weeks of *A. indica* LFF administration were shown in Table 4. There was a significant elevation in blood glucose levels in diabetic group as compared to normal control mice. Administration of glibenclamide (7 mg/kg b.w.) and *A. indica* LFF (500 and 1000 mg/kg b.w.) reduced the blood glucose in diabetic mice as compared to control mice. The 4th week treatment with glibenclamide and 1000 mg/kg b.w. of *A. indica* LFF resulted in significant hypoglycemic effect in diabetic group.

Effect of *A. indica* LFF on serum AST and ALT in alloxan induced diabetic mice

Serum AST and ALT levels in normal and experimental diabetic mice were determined. There was a significant elevation of serum AST and ALT in diabetic control mice as compared to non-diabetic control mice. Administration of glibenclamide (7mg/kg b.w.) and *A. indica* LFF (500 and 1000 mg/kg b.w.) significantly decreased AST and ALT levels in diabetic mice as compared to diabetic control mice (Table 5).

Effect of *A. indica* LFF on serum serum lipid profiles in alloxan induced diabetic mice

Serum cholesterol and triglyceride levels were determined in normal and experimental diabetic mice. Administration of glibenclamide (7mg/kg b.w.) and *A. indica* LFF (500 and 1000 mg/kg b.w.) significantly decreased the serum cholesterol and tryglyceride levels in diabetic mice as compared to diabetic control mice (Table 6).

Table 1: Total phenolic and flavonoid content in *A. indica* leaf flavonoid fraction (LFF)

Total phenolic content	328 mg GAE/g
Total flavonoid content	241 mg ChE/g

Table 2: **Effect of CFF in Oral Glucose Tolerance Test in normal fasted mice**

Sl. No.	Group	Blood Glucose level mg/dl (mean±SD)				
		Fasting	Post treatment			
		0	30 min	60 min	120 min	150 min
1	Control (PBS)	74.35±2.42	78.43±2.69	76.15±2.92	75.42±2.79[a]	75.42±2.45[a]
2	Glibenclamide (7mg/kg)+ glucose	78.23±2.87	200.7±3.06	154.48±2.45	101.34±3.02[b]	81.57±2.58[a]
3	CFF (500 mg/kg)+ glucose	78.99±2.61	201.44±2.78	161.59±3.25	104.74±3.25[b]	95.58±2.91[b]
4	CFF (1000 mg/kg)+ glucose	72.46±2.75	194.9±2.76	153.64±2.84	94.89±3.24[b]	80.08±2.59[a]

Values are expressed as mean±SD. ANOVA followed by Duncan's multiple range test. Values not sharing a common superscript differ significantly.

Table 3: **Effect of CFF on blood glucose level in normal fasted mice**

Sl. No.	Group	Blood glucose level mg/dl (mean±SD)			
		Fasting	Time (h) after treatment		
			1	2	4
1	Control (PBS)	73.87±2.35	71.93±2.66	70.73±2.45	73.34±2.63[a]
2	Glibenclamide (7mg/kg)+ glucose	77.99±2.54	68.17±2.67	60.11±2.47	56.42±2.62[b]
3	CFF (500 mg/kg)+ glucose	71.94±3.01	70.11±2.49	63.99±2.68	60.1±2.6[c]
4	CFF (1000 mg/kg)+ glucose	76.86±3.04	66.3±2.57	59.43±2.68	54.02±2.73[b]

Values are expressed as mean±SD. ANOVA followed by Duncan's multiple range test.
Values not sharing a common superscript differ significantly.

Table 4: **Effect of CFF on blood glucose level in alloxan induced diabetic mice**

Sl. No.	Group	Blood Glucose Level mg/dl (mean±SD)				
		Base	Weeks after treatment			
			1	2	3	4
1	Control (PBS)	74.25±2.95	75.7±4.29	73.83±3.04	73.18±3.22	72.53±3.4[a]
2	Diabetic control (alloxan)	206.57±4.26	205.82±3.51	212.29±3.03	210.48±3.08	208.67±3.13[b]
3	Diabetic+Glibenclamide (7mg/kg)	203.67±2.74	188.24±2.66	175.82±5.11	168.92±4.82	162.02±4.54[c]
4	Diabetic+CFF (500 mg/kg)	205.17±2.96	189.16±3.08	181.21±4.3	170.3±3.96	159.4±3.63[c]

5	Diabetic+CFF (1000 mg/kg)	204.55±2.75	182.96±3.64	167.6±3.7	160.2±3.42	152.81±3.15[d]

Values are expressed as mean±SD. ANOVA followed by Duncan's multiple range test. Values not sharing a common superscript differ significantly.

Table 5:**Effect of CFF on serum AST and ALT in alloxan induced diabetic mice**

Sl. No.	Group	AST (IU/L)	ALT (IU/L)
1	Control (PBS)	50.82±4.17[a]	57.33±5.06[a]
2	Diabetic control (alloxan)	192.51±7.25[b]	190.93±4.21[b]
3	Diabetic+Glibenclamide (7mg/kg)	80.83±4.36[c]	89.57±4.64[c]
4	Diabetic+CFF (500 mg/kg)	121.55±4.6[d]	122.37±4.63[d]
5	Diabetic+CFF (1000 mg/kg)	83.62±4.4[c]	91.84±3.67[c]

Values are expressed as mean±SD. ANOVA followed by Duncan's multiple range tests. Values not sharing a common superscript differ significantly.

Table 6: **Effect of CFF on serum Cholesterol and triglycerides in alloxan induced diabetic mice**

Sl. No.	Group	Cholesterol (mg/dl)	Triglycerides (mg/dl)
1	Control (PBS)	130.93±7.94[a]	151.61±5.49[a]
2	Diabetic control (alloxan)	262.6±8.51[b]	237.27±5.76[b]
3	Diabetic+Glibenclamide (7mg/kg)	133.93±6.82[a]	165.57±5.98[c]
4	Diabetic+CFF (500 mg/kg)	162.56±9.51[c]	188.8±7.91[d]
5	Diabetic+CFF (1000 mg/kg)	146.27±6.31[d]	170.47±4.27[c]

Values are expressed as mean±SD. ANOVA followed by Duncan's multiple range test. Values not sharing a common superscript differ significantly.

DISCUSSION

The basic mechanism behind hyperglycemia includes over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues (Latner, 1958). Persistent hyperglycemia can cause most diabetic complications (American Diabetes Association, 1998).

In the present study, results of oral glucose tolerance test and normoglycaemic (euglycemic) studies revealed that, *A. indica* LFF has the capacity to lower blood glucose levels. The diabetic syndrome in mice administered with alloxan is characterized by stable-moderate hyperglycemia, glucose intolerance and altered but significant glucose stimulated insulin secretion (Masiello et al., 1998). Alloxan induced diabetic mice administered with *A. indica* LFF (500 mg/kg b.w.) showed 7.8, 11.6, 16.9 and 22.3% decline respectively in the blood glucose levels. Whilst a decline of 10.5, 18.06, 21.6 and 25.2% were observed in animals treated with 1000 mg/kg b.w. of the *A. indica* LFF *A. indica* on days 7, 14, 21 and 28 respectively.

Theoretically, hypoglycemic plants act through a variety of mechanisms. There are some medicinal plants which are known to exert antidiabetic activity without the stimulation of insulin secretion (Hannan et al., 2003; Maghrani et al., 2003; Sachdewa and Khemani, 2003). At present juncture, it is not possible either to pin point the exact mechanism of the antidiabetogenic effect of the *A. indica* LFF to identify the active principle(s) responsible for such effect. Nevertheless, some hypothetical suggestions could be made. Plant polyphenols, such as green-tea catechins (Matsumoto et al., 1993), persimmon-leaf polyphenols (Kawakami et al., 2010) and apple-leaf extract (Shirosaki et al., 2012) inhibit increases in blood-glucose levels. This phenomenon has been attributed to the promotion of insulin secretion (Anderson and Polansky, 2002).

The activities of serum transaminases (AST and ALT) are elevated in diabetic mice (Ghosh and Suryawanshi, 2001). The increased gluconeogenesis and ketogenesis observed in diabetes may be due to the high levels of these transaminases (Felig et al., 1970). The restoration of AST and ALT activities to their respective normal levels after supplementation of *A. indica* LFF further strengthens the anti-diabetogenic effect of LFF. Moreover, AST and ALT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver.

The common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Khan et al., 1995; Mitra et al., 1995). Hypertriglyceridemia is also associated with metabolic consequences of hypercoagulability,

hyperinsulinemia, insulin resistance and insulin intolerance (Gingsberg, 1994). In the present study, administration of LFF to the alloxan induced diabetic mice significantly ($p < 0.05$) improved the cholesterol and triglyceride profiles by reducing their levels (Table 6). The observed hypolipidaemic effect of *A. indica* LFF may be because of decreased cholesterogenesis and fatty acid synthesis. Various studies on medicinal plants have reported a similar lipid lowering activity (Ram et al., 1997; Sharma et al., 1997; Jouad et al., 2003).

CONCLUSION

The significant antidiabetic activity of the *A. indica* LFF in the present study could be attributed to its flavonoid content. Studies are in progress in our laboratory to elucidate the molecular and cellular mechanisms of *A. indica* LFF and its principle constituents. Longer duration studies on chronic models may contribute toward the development of a potent antidiabetic drug.

ACKNOWLEDGMENTS

Financial support from Department of Atomic Energy (DAE), Board of Research in Nuclear Sciences (BRNS), Government of India (No. 2009/34/27/BRNS/1995; dt.: 22-12-2009) in the form Research Project to Vadlapudi Kumar is gratefully acknowledged. Authors are thankful to Spectroscopy/Analytical Test facility, Indian Institute of Science, Bangalore, India for the GC-MS analysis and Dr. Kishore Bhat, Department of Microbiology, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum, Karnataka, India for the human cell culture studies (cytotoxicity assays).

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