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Hypoglycemic Activity of *Lepidium sativum* Linn Seed Total Alkaloid on Alloxan Induced Diabetic Rats

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ABSTRACT

Lepidium sativum Linn. (Family Cruciferae) popularly known as Garden cress has been used in traditional and folklore medicine for the treatment of bronchial asthma, diabetes, local and rheumatic pain. The present study aimed at investigation of antidiabetic efficacy of *L. sativum* Seed Total Alkaloid (LSTA). The major components of this alkaloid fraction are lepidine and semilepidine, a rare group of imidazole alkaloid. Antidiabetic profile of LSTA (50, 150 and 250 mg kg^{-1}, i.p.) was assessed on alloxan induced diabetic rats upon 21 days continuous treatment. Biochemical parameters viz., glucose, total cholesterol, triglycerides, low density lipoprotein, high density lipoprotein, urea and creatinine were determined along with b.wt. and relative organ weight. LTSA at 250 mg kg^{-1} showed 1.94% b.wt. gain on 21th day relative to 6.14 and 8.94% of control and diabetic group. LSTA at 250 mg kg^{-1} dose significantly (p<0.001) suppressed blood glucose, cholesterol, triglyceride and urea level in diabetic rats. The results revealed that LSTA at dose 250 mg kg^{-1} showed potent hypoglycemic activity. The *L. sativum* alkaloid have potential antidiabetic effect against alloxan-induced diabetes may be through reducing oxidative damage and modulating antioxidant enzymes. The possible mechanism by which LSTA brings about its anti-hyperglycaemic action may be by potentiation of pancreatic secretion of insulin from the remaining islet β cells.

Key words: *Lepidium sativum*, seed, imidazole alkaloid, hypoglycaemic, alloxan

INTRODUCTION

Worldwide diabetes mellitus has reached epidemic proportions as per the World Health Organization (WHO) which is strongly related to lifestyle and economic changes. Over the next decade the number of diabetic patient worldwide will exceed the figure of 200 million. By the year 2025, India is predicted to have the most number of people with Diabetes Mellitus (DM) in the world (Sridhar, 2000). Diabetes requires continuing medical care to prevent acute complications and the risk of long-term complications. Diabetes care is complex and requires a range of interventions along with glycemic control to improve outcomes (ADA, 2009).

Many diabetic people turn to complementary therapies to control the chronic nature and threat to quality of life eventually reducing the complications (Israili *et al.*, 2007). For a long time plants based herbal medicines have been the major source of drugs for treatment of DM in Ayurveda and other ancient systems of medicine (Akhtar and Ali, 1984), as plant products are generally considered to be less toxic and free from side effects compared to modern synthetic drugs

(Brinker, 1988). Ethnobotanical information refers to suggestive antidiabetic potential in about 800 medicinal plants (Ilango and Chitra, 2009) and many herbs have been reported to possess hypoglycemic activity in the literature (Pepato *et al.*, 2003). Investigations on more effective and safer hypoglycemic agents from medicinal plants has continued to be an important area of active research after WHO's promotion of traditional herbal therapies.

Lepidium sativum is commonly known as chandrasura belonging to family Cruciferae popularly known as Garden cress in English. *L. sativum* is a small, annual, herbaceous plant of 15-45 cm height, cultivated throughout India. The reddish coloured seeds are oblong, angular, slightly curved on one side having rugous surface. Seeds are odorless, pungent and mucilaginous used as salad supplement. The seeds are used in chronic enlargement of liver and spleen, as carminative adjunct to purgatives, in skin diseases, dysentery, diarrhoea, asthma and in liver complaints (Khory, 1999; Kirtikar and Basu, 2006; The Wealth of India, 1962). Literature search revealed tachyphylactic (Vohra and Khan, 1977), diuretic (Navarro *et al.*, 1994), oral contraceptive (Sharief and Gani, 2004) antihypertensive (Maghrani *et al.*, 2005) effect of *L. sativum* seeds aqueous extract. The aqueous extract of *L. sativum* seed showed hypoglycemic activity in human subjects and are used commonly in the treatment of bronchial asthma (Eddouks *et al.*, 2002; Archana and Mehta, 2006).

Preliminary phytochemical study of *L. sativum* following standard procedure showed presence of flavonoids, coumarins, sulphur containing glycosides, triterpenes, sterols and various imidazole alkaloids (Patel *et al.*, 2009). The major secondary metabolites of this plant are glucosinolates (Gill and MacLeod, 1980). *L. sativum* contain rare imidazole alkaloids known as lepidine and semilepidine (Maier *et al.*, 1998). From methanolic extract of defatted seeds sinapic acid and sinapin were isolated (Nayak *et al.*, 2009). Despite the wide spread traditional and edible uses of *L. sativum*, very few pharmacological studies have been done so far. Phytopharmacological screening of alkaloid and glucosinolates are untouched so far. The present investigation was undertaken to screen the hypoglycemic effect of the total alkaloid of *L. sativum*.

MATERIALS AND METHODS

Collection and identification of plant material: The seeds of *L. sativum* were purchased from local market of Bhopal, Madhya Pradesh, India in Sept 2010. The seeds were taxonomically identified by Dr. H.B. Singh, Scientist, NISCAIR, New Delhi, India. A voucher specimen was deposited in the herbarium of NISCAIR (*L. sativum*; No. NISCAIR/RHMD/Consult/-2009-10/1232/36).

Extraction of total alkaloid: Coarsely grounded seeds (1.5 kg) were defatted with n-hexane for 16 h in a Soxhlet extractor and subsequently extracted with methanol for 8 h. The resulting methanolic extract was evaporated to dryness, resuspended in water and acidified with concentrate hydrochloric acid. This mixture was extracted three times with ethyl acetate, the remaining aqueous layer was basified with concentrate ammonia and extracted again three times with ethyl acetate. Both the separated ethyl acetate layer was combined, concentrated under reduced pressure and dried. This final extract gave positive result for Dragendorff test (Kokate *et al.*, 2005) and designated as *L. sativum* total alkaloid (LSTA).

Test animal: Laboratory bred female Albino mice (20-25 g) and Wistar albino rat (120-150 g) of either sex were maintained under standard laboratory conditions at 22±2°C, relative humidity

50±15% and photoperiod (12 h dark and light), were used for the experiment. Commercial pellet diet (Hindustan Lever, Mumbai, India) and water were provided *ad libitum.*

Ethical clearance: All studies were carried out in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India. Institutional Animal Ethical Committee approval (approval No. IAEC/RCP/2008/05) was obtained before conduction of experiments.

Acute toxicity test: Acute oral toxicity of LSTA was determined following the Up and Down (OECD guideline No. 425) and Fixed dose method (OECD guideline No. 420) of Organization for Economic Co-operation and Development (OECD). A limit test was performed to categorize the toxicity class of the compound followed by main test to estimate the exact LD_{50} on healthy female albino mice (20-25) as per guidelines. Animals were observed continuously up to 4 h for detailed behavioral and autonomic profiles. Signs of delayed toxicity or mortality were recorded up to a period of fourteen days (OECD, 2000).

Anti-diabetic activity
Induction of diabetes in rats: Diabetes was induced on overnight fasted rats by a single dose of freshly prepared alloxan monohydrate (150 mg kg^{-1}, i.p.) in normal saline (Etuk and Muhammed, 2010). Blood Glucose (BG) level was measured by using one-touch glucometer and diabetes was confirmed after 72 h of alloxanisation. Rats with fasting BG level more than 150 mg dL^{-1} were considered to be diabetic and were selected for studies.

Study protocol: Rats of either sex were divided randomly six per group. Group I was vehicle control, Group II standard drug treated group and Group III-V was LSTA treated, respectively in 50, 150 and 250 mg kg^{-1} doses consecutively for 21 days. Dried alkaloidal extract was insoluble in water thus mixed with few drops of tween 80 then suspended in distilled water and used as vehicle control. During the study period body weight and BG level were recorded at 1st, 7, 14 and 21st day. On the 21st day animals were sacrificed and organs (liver, pancreas and kidney) were isolated and kept in ice cold saline solution, blotted and weighed. Blood samples collected by heart puncture, serum was separated after coagulating at 37°C for 30 min and centrifuged at 3000 rpm for 10 min. Serum was analysed for various biochemical parameters Total Cholesterol (TC), triglycerides (TG), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), urea and creatinine.

Biochemical estimations: Serum content of glucose, TC, TG, HDL, urea and creatinine were estimated in Span Auto analyzer (India) using commercial kit (Span Diagnostics Pvt. Ltd., India). Serum LDL cholesterol content was calculated with formula using serum concentration values of total cholesterol, triglyceride and HDL cholesterol.

Statistical analysis: Experimental data were analyzed using one way ANOVA followed by Tukey-Kramer multiple comparison test. The p-value less than 0.05 were considered statistically significant. Graph Pad Prism Version 3.02 was used for statistical calculations.

RESULTS
Acute toxicity test: Based on the OECD guidelines a Limit test was performed at 2000 mg kg^{-1} (i.p.) which showed mortality (40.0%). A main test was performed to determine the exact LD_{50} value following OECD up and down method. LD_{50} of LSTA was calculated to be 2204.95 mg kg^{-1} from

graphical representation. The test substance could be classified in the hazard classification as Class 4, 300 mg kg^{-1} <LD_{50}<2000 mg kg^{-1} in the Globally Harmonized System (GHS). A dose range of 50, 150 and 250 mg kg^{-1} was selected for evaluation of pharmacological activity.

Effect on body weight: Body weight was determined on zero day then on 7, 14 and 21th day, respectively. Weight gain in vehicle control group during the 7, 14 and 21th day was 2.07, 4.21 and 6.14%, respectively. Diabetes prevents the weight gain on 14 and 21th day by 7.38 and 8.94%, respectively. Standard drug metformin treated group and LTSA (50, 150 and 250 mg kg^{-1}) treated groups showed decrease in body weight gain by 2.41, 4.95, 3.02 and 1.94% on 21th, respectively (Table 1).

Effect on liver, kidney and pancreas weight: Liver kidney and pancreas weight were determined on 21st day after sacrifice of animals. The increase in weight of liver was highly significant (p<0.001) in diabetic animals compared with vehicle control group. All the doses of LSTA significantly (p<0.001) decreased liver weight in diabetic animals. The weight of kidney was highest in vehicle control group and lowest in diabetic control group. LSTA at dose of 250 mg kg^{-1} showed weight of kidney 0.759±0.028 where as standard drug showed 0.768±0.042 per 100 g b.wt. The pancreas weight was increased high significantly in diabetic group when compared with control group. LSTA (50 and 150 mg kg^{-1}) effectively suppressed (p<0.001) pancreas weight in comparison to diabetic control group (Table 1).

Effect on serum blood glucose: Fasting BG levels were determined on day 1st and every 7th day for three weeks (Table 2). BG in the diabetic group increased gradually from the 1st day to the termination of experimental period (p<0.001) in comparison to vehicle control group. LSTA (150 and 250 mg kg^{-1}) effectively suppressed (p<0.001) the increase of BG level in comparison to diabetic control group on 7th day onward. Metformin treated groups significantly suppressed (p<0.001) serum BG level after 14th day to the termination of experimental protocol.

Table 1: Effects of *Lepidium sativum* total alkaloidal treatment on body weight of alloxan induced diabetic rats

| Treatment (mg kg^{-1}) | Body weight | | | | Weight (g/100 g b.wt.) | | |
| | Average weight in g (% Change) | | | | | | |
	0 Day	7th Day	14th Day	21st Day	Liver	Kidney	Pancreas
Vehicle control	140.06±18.61	142.92±19.76 (+2.07%)	145.92±21.34 (+4.21%)	148.63±18.46 (+6.14%)	2.485±0.021	0.812±0.032	0.284±0.021
Diabetic control	147.52±16.45	150.81±19.56 (+1.55%)	136.60±16.29 (-7.38%)	134.38±15.47 (-8.94%)	4.342±0.035[a]	0.680±0.060[ns]	0.549±0.042[a]
Metformin (20)	145.02±18.65	154.32±20.41 (+6.41%)	148.65±18.85 (+2.48%)	141.57±14.41 (-2.41%)	2.847±0.091***	0.768±0.042[ns]	0.409±0.011**
LSTA (50)	157.58±20.76	173.22±22.72 (+9.96%)	158.55±20.51 (+0.63%)	149.72±16.78 (-4.95%)	3.982±0.078***	0.688±0.025[ns]	0.346±0.014***
LSTA (150)	168.73±18.98	183.68±23.89 (+8.83%)	170.87±19.78 (+1.24%)	163.60±16.76 (-3.02%)	2.867±0.030***	0.745±0.021[ns]	0.358±0.018***
LSTA (250)	190.05±22.54	205.59±24.62 (+8.15%)	193.59±20.49 (+1.84%)	186.31±12.45 (-1.94%)	2.564±0.022***	0.759±0.028[ns]	0.398±0.023**

The values are expressed as Mean±SEM, n: 6 in each group. a: p<0.001 compared to vehicle control group, ***p<0.001, **p<0.01 and ns: Non-significant compared with Diabetic control group. Values in parenthesis signify percentage change in body weight compared to initial body weight (0 day)

Table 2: Effects of *Lepidium sativum* total alkaloidal treatment on blood glucose level of alloxan induced diabetic rats

Treatment (mg kg^{-1})	Day 0	Day 7	Day 14	Day 21
Vehicle control	82.52±3.84	80.33±6.41	84.62±5.41	83.70±3.11
Diabetic control	277.25±18.41[a]	282.50±16.24[a]	284.51±19.41[a]	278.45±18.74[a]
Metformin (20)	288.60±17.24[ns]	232.61±15.87[ns]	185.48±14.57***	98.47±13.46***
LSTA (50)	244.53±15.57[ns]	252.42±18.46[ns]	248.73±16.28[ns]	224.22±14.23[ns]
LSTA (150)	226.87±16.48[ns]	205.37±12.33**	175.76±16.37***	126.46±11.42***
LSTA (250)	218.49±14.76[ns]	196.30±13.42**	142.22±14.68***	124.40±12.23***

The values are expressed as Mean±SEM, n: 6 in each group. a: $p<0.001$ compared to vehicle control group, ***$p<0.001$, **$p<0.01$ and ns: Non-significant compared with diabetic control group

Table 3: Effects of *Lepidium sativum* total alkaloidal treatment on serum biochemical parameter of alloxan induced diabetic rats

Treatment (mg kg^{-1})	Serum biochemical parameters					
	TC	TG	LDL	HDL	Urea	Creatinine
Vehicle control	98.22±2.94	98.50±1.30	55.46±1.62	22.05±0.55	32.09±1.10	0.62±0.04
Diabetic control	149.16±4.40[a]	149.16±4.22[a]	141.81±10.46[a]	16.85±1.20[ns]	64.63±1.11[a]	1.43±0.03[c]
Metformin (20)	86.53±4.20***	80.39±9.23***	50.42±2.72***	26.24±1.14**	35.97±1.54***	0.64±0.01*
LSTA (50)	79.31±3.72***	71.73±4.45***	31.18±3.89***	36.81±1.96***	61.30±1.58[ns]	1.23±0.02[ns]
LSTA (150)	69.55±0.86***	62.32±2.57***	20.70±1.64***	39.09±2.73***	46.08±1.77***	0.86±0.02[ns]
LSTA (250)	61.71±2.17***	48.31±7.48***	10.92±1.12***	42.02±0.52***	39.33±0.85***	0.74±0.05[ns]

The values are expressed as Mean±SEM, n: 6 in each group. a: $p<0.001$ and c: <0.05 compare to vehicle control group. ***$p<0.001$, **$p<0.01$, *$p<0.05$ and ns: Non-significant compared with Diabetic Control group. TC: Total cholesterol, TG: Triglyceride, LDL: Low density lipoprotein and HDL: High density lipoprotein

Effect on biochemical parameters: Alloxan induced diabetes has significantly increased the serum level of TC, TG, LDL and urea in animals compared to vehicle control group. LSTA at 150 and 250 mg kg^{-1} dose significantly ($p<0.001$) reduced levels of cholesterol, triglyceride and urea in diabetic rats. Effect of LSTA in reducing creatinine level was non-significant in all doses in comparison to diabetic control group. *L. sativum* alkaloid has very prominently ($p<0.001$) increased the level of protective high density lipoprotein in all the tested doses (Table 3).

DISCUSSION

This study compiles the effect of LSTA on several parameters alloxan induced diabetes profile. The percentage yield of total alkaloid was estimated to be 0.29% w/w in the investigated seed variety. The major components of LSTA are rare imidazole alkaloids lepidine and semilepidine having active moiety 2-benzyl imidazole or imidazoline.

LD_{50} of LSTA was found to be 2204.95 mg kg^{-1} intraperitoneally suggesting relatively low toxicity. In the present study, the antidiabetic effect of *L. sativum* total alkaloid has been evaluated at 50, 150 and 250 mg kg^{-1} (i.p.) doses.

Common endocrine disorder DM is defined as a state of chronic hyperglycemia either may be due to the absolute lack of insulin or to factors that oppose its action (Nishikawa *et al.*, 2000). Alloxan induced DM in rats causing destruction of insulin-producing β-cells is widely used as a model of type I insulin dependent DM (Oi *et al.*, 1997; Murata *et al.*, 1998). Decreased serum concentration of antioxidant enzymes were observed in alloxan treated diabetic rats due to their increased utilization during inhibition or destruction of free radical species which also indicates an imbalanced Reactive Oxygen Species (ROS) production and antioxidant scavenging systems.

Several related metabolic disturbances in this kind of diabetes are brought about by increased Oxidative Stress (OS), defects in antioxidant protection (Martinez-Cayuela, 1995) and nonenzymatic glycation of proteins. Reactive Oxygen Species (ROS) attack unsaturated fatty acids of membrane phospholipids to initiate lipid peroxidation causing severe damages of the membrane structure with sequential variation in its fluidity and ability to function correctly (Nishikawa *et al.*, 2000). Increased serum concentration of SGPT, SGOT and ALP were observed in alloxan induced diabetic rats indicating an altered liver function and/ or liver mitochondrial injury in comparison to normal control rats as reported by Lanjhiyana *et al.* (2011). Insulin deficiency contributes to increased serum level of transaminase enzymes due to easy availability of amino acids which leads to enhanced occurrence of gluconeogenesis and ketogenesis processes during diabetes inducing hyperlipidemia and hyperglycemia.

Serum concentration of TC, TG, LDL were increased markedly along with a decreased HDL was observed in diabetic rats compared to normal vehicle control animals. This hyperlipidemic condition certainly contributes to a major risk factor for atherosclerosis and cardio vascular diseases (Nikkila and Kekki, 1973). The saturated fatty acids present in fat could increase the production of TG and cholesterol by the liver and could also decrease the catabolism of LDL-c by the repression of their receptors (Pereira *et al.*, 1990). Insulin deficiency in diabetes contributes to derangements of various metabolic and regulatory mechanisms in body. Insulin deficiency inactivates the lipoprotein lipase promoting conversion of free fatty acids into phospholipids and cholesterol in liver and finally it got discharged into blood resulting into elevated serum phospholipid level (Pushparaj *et al.*, 2007). We found that LSTA treatment significantly reduced TC, TG and LDL levels as well as at the same time raised HDL level more than that of control at 250 mg kg^{-1} upon 21 days administration repeatedly.

Alloxan produces a decrease in the activity of the antioxidant enzymes during the development of alloxan-induced type I DM in liver, pancreas and testis as reported by Soto *et al.* (1998). El-Missiry and El Gindy (2000) observed that alloxan administration produced a significant decrease in hepatic glutathione content and SOD activity accompanied by a significant increase in aldehydic products of lipid peroxidation, indicating an increased hepatic oxidative stress which may also occur in other tissues in alloxan-treated rats. This may contribute to the disruption of intracellular and membrane redox state of many cells including liver and β-pancreatic cells, hence disturbing glucose regulation. The protective effect of *L. sativum* alkaloid may be due to modulation of redox state of liver cells as well as other important secretory cells such as β-pancreatic cells inhibiting the damage produced by the generation of free radicals.

Administration of LSTA effectively prevented the increase in BG levels without causing a hypoglycaemic state may be due to restoration of the delayed insulin response and slow absorption of carbohydrate (glucose). *L. sativum* may possess insulin-like activity helpful to reduce the incidence of lipid born complications. It is suggested that the active principles from plant sources might act by several mechanisms such as stimulating insulin secretion, increasing repair/proliferation of β-cells, enhancing the effect of insulin and adrenaline and increasing the antioxidative capability (Shanmugasundaram *et al.*, 1990). The possible mechanism by which LSTA brings about its anti-hyperglycemic action may be by potentiation of pancreatic secretion of insulin from the remaining islet β cells. Other probable mechanisms by which the alkaloid of *L. sativum* lowered blood glucose levels in diabetic rats might be by increasing glycogenesis, inhibiting gluconeogenesis in the liver, or inhibiting the absorption of glucose from the intestine or these might have improved insulin resistance (Eddouks *et al.*, 2002). Garden cress (*L. sativum*)

seeds showed reduction in starch hydrolysis (41%) of starch to glucose in diabetic subjects and were tested in vivo on 11 non insulin dependent diabetes mellitus subjects as well as 14 normal healthy subjects. In the 21 days treatment of diabetics with 15 g day^{-1} of *L. sativum* 9 out of 11 subjects showed reduction in the levels of blood glucose from 10.2 mM L^{-1} at the end of study period indicating its potential of acting a hypoglycemic agent (Patole *et al.*, 1998). Ethnopharmacological survey in south-eastern Morocco revealed use of *L. sativum* for management of DM with an average citation of 64 among the 500 persons interviewed (Eddouks *et al.*, 2002). Present findings are in agreement with previous studies carried out by Eddouks *et al.* (2005) with aqueous seed extract (20 mg kg^{-1}) of *L. sativum* on normal and streptozotocin-induced (STZ) diabetic rats producing a significant decrease on blood glucose levels after daily repeated oral administration for 2 weeks. Eddouks and Maghrani (2008) assessed the effect of *L. sativum* on renal glucose reabsorption and urinary TGF-β 1 levels of diabetic rats. Intravenous perfusion of *L. sativum* aqueous extract (10 mg kg^{-1} h^{-1}) in normal and streptozotocin-induced diabetic rats at 20 mg kg^{-1} normalized glycaemia and decreased the amount of urinary TGF-β1 in diabetic rats acting as a potent inhibition of renal glucose reabsorption.

Phytochemical constituents such as alkaloids have shown hypoglycemic activity in animal studies (Karawya *et al.*, 1984). The leaves and stems of *Catharanthus roseus* are the sources of dimeric alkaloids, vincristine and vinblastine, used traditionally in various regions of the world including India, West Indies as well as Nigeria to control diabetes (Cowley and Bennett, 1928). Fresh leaf juice of *C. roseus* has been reported to reduce blood glucose in normal and alloxan diabetic rabbits (Nammi *et al.*, 2003) and have hypoglycaemic activity in streptozotocin induced diabetic rats (Singh *et al.*, 2001). Ahmed *et al.* (2010) reported anti-hyperglycemic activity of *Vinca rosea* alcoholic extract in alloxan-induced hyperglycemic rats by acting on pancreatic β-cell regeneration. *C. roseus* showed rapid normalization of blood glucose levels in streptozotocin-treated animals could be due to insulin releasing effect of hypoglycemic alkaloids like catharanthine, leurosine, lochnerine, tetrahydro-alstonin, vindoline and vindolinine (Gordon *et al.*, 1964), suggesting binding with insulin receptors to act as insulin secretagogue, like biguanides (Islam *et al.*, 2009). Isoquinoline alkaloid rich fraction derived from stem of *Tinospora cordifolia* significantly decreased gluconeogenesis in rat hepatocytes and increases insulin secretion. Three major alkaloids viz., palmatine, jatrorrhizine and magnoflorine of *T. cordifolia* stimulated insulin secretion from the RINm5F cell line (Patel and Mishra, 2011).

Garden cress is widely used all over the world since ancient times both as nutraceutical and food stuff. *L. sativum* has known to be effective against plethora of diseases especially DM. Though number of studies has been performed in animals and human for assessing hypoglycemic activity, attempts has not been directed towards phytopharmacological mechanistic studies for identification of bioactive compounds. This study was conceptualized to identify the role of imidazole alkaloids in its antidiabetic potentials. The *L. sativum* alkaloid have potential antidiabetic effect against alloxan-induced diabetes may be through reducing oxidative damage and modulating antioxidant enzymes by dose dependent manner (Shinde *et al.*, 2010).

Imidazoles are important class of heterocyclic having broad applications in clinical therapies as antibacterial, antifungal, anti-inflammatory and histamine agonist etc. (De Luca, 2006). Large number of highly significant biomolecules such as the essential amino acids, biotin and some plant alkaloids have imidazole nucleus. Crane *et al.* (2006) reported potent effect of nine synthetic compounds with methyl substituted imidazoline moiety on the glucose tolerance in normal and STZ-diabetic rats. Koistinen *et al.* (2003) highlighted the potential importance of AMP-activated protein

kinase-dependent pathways (AMPK) in the regulation of GLUT4 and glucose transport activity in insulin-resistant skeletal muscle by 5-amino-imidazole carboxamide riboside. Activation of AMPK increases glucose transport via an insulin-independent mechanism by increased regulation of cell-surface GLUT4 content in insulin resistant skeletal muscle.

Isolation and identification of bioactive alkaloid and establishment of exact mechanism of action is to be carried out. Further studies are under progress to confirm the involvement of alkaloid and its mechanism of antidiabetic activity.

CONCLUSION

The results of the present study demonstrate antidiabetic activity of *L. sativum* total alkaloid. *L. sativum* alkaloid belongs to imidazole category with poly substituted phenyl- imidazoline basic nucleus. The exact mechanism of action for interaction with glucose utilization or β-cytotropic activity remains to be elucidated. Further studies on antihyperglycemic and antihyperlipidemic profile of the alkaloid are going on in our laboratory to explore exact mode of action on specific interaction with glucose metabolism.

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