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Phytochemical and CNS activity of *Lepidium Sativum* Linn. seeds total alkaloid

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ABSTRACT

Lepidium sativum Linn. has been used in traditional and folklore medicine for the treatment of bronchial asthma, diabetes, local and rheumatic pain. The present study deals with the investigation of the neurobehavioral effects of the total alkaloid from seeds of Lepidium sativum [LSAF].The effect of LSAF on general pharmacology, thiopental induced hypnosis, locomotor activity, motor coordination, antianxiety and analgesic effect were studied. The animals were intraperitonealy treated with 50, 150 and 250 mg/kg LSAF to the respective treatment groups. The results revealed that LSAF considerably potentiated the thiopental induced hypnosis, decreased locomotor activity and motor coordination, and increased preference to plus maze open arm. LSAF also increase the reaction time in caudal immersion and decrease in number of writhes in acetic acid induced writhing. It has been concluded that LSAF exhibited sedative, anxiolytic, myorelaxant and analgesic activity.

Key words: *Lepidium sativum*, imidazole alkaloid, analgesic, sedative, antianxiety.

INTRODUCTION

Lepidium sativum (*L. sativum*) is commonly known as chandrasura. This is a small, herbaceous, glabrous annual, 15-45 cm high plant cultivated as salad supplement throughout India. The seeds are reddish in colour, oblong, somewhat angular and curved slightly on one side with rugous surface. Near the point of attachment there is a white scar, from which a small channel extends to 1/3 the length of the seeds. Seeds are odorless and taste is pungent and mucilaginous [1].

Cold infusions of seeds are used to relieve hiccough. The seeds are used in chronic enlargement of liver and spleen and also used as carminative adjunct to purgatives. The bruised seeds, mixed with lime juice are used as local application for the relief of inflammatory and rheumatic pains. The seed are bitter, thermogenic, depurative, rubefacient, galactagogue, emmenagogue, tonic,

aphrodisiac and diuretic. They are useful as poultices for sprains, and in leprosy, skin diseases, dysentery, diarrhoea, splenomegal and asthma [2]. The leaves are mild stimulant and diuretic, useful in scorbutic diseases and in liver complaints [3].

Literature search revealed that the *Lepidium sativum* has tachyphylatic and diuretic action [4-5]. *Lepidium sativum* aqueous extract has oral contraceptive and antihypertensive effect [6-7]. The seeds aqueous extract showed hypoglycemic activity and used in the treatment of bronchial asthma [8-9].

L. sativum have been widely used to treat a number of ailments in traditional system of medicine throughout India. Preliminary phytochemical study of *L. sativum* following standard procedures showed presence of flavonoids, coumarins, sulphur glycosides, triterpenes, sterols and various imidazole alkaloids [10]. The major secondary compounds of this plant are glucosinolates [11]. The alkaloids of *L. sativum* are member of the rare imidazole alkaloids that is known as lepidine [12]. Despite the widespread traditional/edible uses of *L. sativum*, there is very few pharmacological works done. Phytopharmacological screening of alkaloid and glucosinolates are untouched so far. The present investigation was initiated to screen the neuropharmacological profile of the alkaloid of *L. sativum.*

MATERIALS AND METHODS

Collection and identification of plant material

The seeds of *L. sativum* (Family: Cruciferae) were purchased from local market of Bhopal, Madhya Pradesh, India. 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

Ground seeds (1.5 kg) were defatted with n-hexane in a soxhlet extractor for 16 h and subsequently extracted with methanol for 8 h. The resulting extract was evaporated to dryness, resuspended in water, acidified with concentrate hydrochloric acid and extracted three times with ethyl acetate. The remaining aqueous layer was basified with concentrate ammonia and extracted again three times with ethyl acetate. Ethyl acetate layer was separated, concentrated under reduced pressure and dried. It gave positive result for Dragendorff test [13]. The percentage yield of total alkaloid was 0.29 % w/w.

Materials

The drugs used in this study were gift samples from the following sources - Morphine (Pharma Chemico Lab., Solan) and Diazepam (Ranbaxy, Dewas). All the other chemicals were of analytical grade.

Test Animals

Laboratory bred Swiss Albino mice (20-25 gm) and Wistar albino rats (120-150 gm) 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, India) and water were provided *ad libitum.*

Study Protocol
For all the studies overnight fasted animals (mice or rat) of either sex were divided randomly in six per group. Group I is vehicle control, Group II-IV is LSAF treated respectively in 50, 150 and 250 mg/kg doses and Group V is standard drug treated group. Dried alkaloidal extract was mixed with few drops of tween 80, then suspended in distilled water and same used as vehicle control.

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
Healthy albino mice of either sex (20-25 gm) were subjected to acute toxicity study as per guidelines suggested by the Organization for Economic Cooperation and Development (OECD). The mice were observed continuously up to 4 h for detailed behavioral and autonomic profiles; signs of toxicity or mortality were recorded up to a period of fourteen days [14].

General pharmacological observation on mice
Behavioral effects of alkaloidal extract (50, 150, 250 mg/kg) were assessed by the method described by Irwin *et al*. 1968 [15]. The mice were divided into three groups and treated with alkaloidal extract at a dose of 50, 150 and 250 mg/kg, respectively. The animals were then placed in an observation cage and observed closely up to 2 h for behavioral changes. The observation parameters consisted of alertness, body position, reactivity to touch, auditory stimuli, righting reflex and lacrimation.

Effect on Thiopental-induced hypnosis on mice
After 30 min of intraperitoneal injection of vehicle, different LSAF doses and standard drug diazepam (2mg/kg, i.p.), all groups of animals were treated with thiopental sodium (25 mg/kg, i.p.). Onset of sleep (loss of righting reflux) and duration of sleep (period between loss of righting reflux and its revival) were recorded [16].

Effect on locomotor activity on mice
Locomotor activity was recorded with a digital activity cage (Dolphin, India). The animals were randomly divided into five groups and each mouse was individually placed in the actophotometer for 10 min to score the basal reading. All the animals were treated with vehicle, different doses of alkaloid and standard drug diazepam (2 mg/kg, i.p.). After 30 min they were again placed individually in the actophotometer to score locomotor activity. Mean change in the locomotor activity was calculated for each group [17].

Effect on motor coordination on mice
Digital rotarod apparatus (Jyoti Scientific, Gwalior, India) was used to evaluate the muscle relaxing and sedative effects of LSAF. The animals were placed individually on the rotarod, rotating at a speed of 25 rpm to score the fall off time. Respective groups of animals were treated with vehicle, different doses of LSAF and reference standard diazepam (2 mg/kg, i.p.). All

animals were subsequently assessed for their performance on the rotarod after 30 min of drug treatment and percentage change in fall off time were calculated [18].

Elevated plus-maze test on mice

This test is used as a standard model to assess anxiolytic activity of drugs. Locally fabricated apparatus consisting of two open arms (16 x 5 cm) and two enclosed arms (16 x 5 x 12cm) elevated to the height of 25 cm [19-20] as validated by Lister, 1987 was used [21]. Animals of all five groups were treated with vehicle, different doses of LSAF and reference standard diazepam (2mg/kg, i.p.) respectively, 30 min before the test. The mice were placed individually in the centre of the maze, head facing toward open arm. The number of entries in open and closed arms, and total time spent in open and closed arms respectively, were recorded for a period of 5 min. Entry into an arm was defined as the point when the animal places all four paws onto the arm [22].

Caudal immersion in hot water

The tail of each rat was immersed individually in hot water at temperature 55 ± 0.5°C for a maximum of 20 sec to avoid injury to the animal. The time until the typical reaction, like withdrawal of tail with a violent jerk was recorded to the nearest tenth of a second. After scoring of basal reaction time, all groups of the animals were treated respectively with vehicle, LSAF and standard drug (morphine, 5 mg/kg, i.p.), and reaction time was again noted after 30 min. Percentage change in mean reaction time was calculated [17].

Acetic acid-induced writhing

The analgesic activity of LSAF on acetic acid-induced writhing was screened on rats following method of Witkin et al. [23]. Animals of all groups were given the writhing agent, 3% aqueous acetic acid (2 ml/kg, i.p.) 30 min after vehicle, LSAF and standard drug (morphine, 5 mg/kg, i.p.) administration. Writhing is defined as a stretch, torson or constriction of abdomen and extension or drawing up of a hind leg etc. The writhing episodes occurred up to 10 min after acetic acid administration were counted and percentage protection was calculated.

Statistical Analysis

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

RESULTS AND DISCUSSION

Acute toxicity test

Based on the OECD guidelines a Limit test was performed to categorize the toxicity class of the compound. The limit test was performed at 2000 mg/kg (i.p), which showed 40.0% mortality. A main test was performed to determine the exact LD_{50} value following OECD up and down method. LD_{50} was calculated as 2204.95 mg/kg from graphical representation. A dose range of 50, 150 and 250 mg/kg was selected for evaluation of pharmacological activities.

Table 1. LD_{50} determination of *Lepidium sativum* alkaloidal fraction (Log dose-Probit) on mice

Dose (gm/kg, i.p.)	Log dose	% Mortality	Corrected % mortality	Probit value
LSAF (1.5)	0.1760	0.00	5.00	3.36
LSAF (1.8)	0.2552	20.00	20.00	4.16
LSAF (2.0)	0.3010	40.00	40.00	4.75
LSAF (2.5)	0.3979	66.66	66.66	5.43

General Pharmacological observation

The LSAF extract at dose of 250 mg/kg showed decrease in alertness, reactivity to touch and auditory stimuli. Lacrimation was absent in all doses of LSAF, while in vehicle treated group all the parameters were normal.

Table 2. Effect of *Lepidium sativum* alkaloidal fraction on general behavior of mice

Dose (mg/kg, i.p.)	Parameters					
	Alertness	**Body position**	**Reactivity to touch**	**Auditory stimuli**	**Righting reflex**	**Lacrimation**
Vehicle	N	N	N	N	N	A
LSAF (50)	N	N	N	N	N	A
LSAF (150)	N	N	N	N	N	A
LSAF (250)	R	N	R	R	N	A

N: Normal, R: Reduced, A: Absent

Effect on Thiopental-induced hypnosis

The LSAF significantly potentiated the thiopental sodium-induced sleeping time in a dose dependent manner. The potentiation in sleeping time were extremely significant ($p<0.001$) in all dose level of LSAF.

Table 3. Effect of *Lepidium sativum* alkaloidal fraction on thiopental (25 mg/kg) induced hypnosis of mice

Dose (mg/kg, i.p.)	Onset of action in min [M ± SEM]	Average sleeping time in min	% Increase in sleeping time
Vehicle	3.33 ± 0.88	14.50 ± 0.91	-
LSAF (50)	2.33 ± 0.33	15.00 ± 1.73***	3.44
LSAF (150)	2.66 ± 0.33	16.00 ± 1.76***	10.34
LSAF (250)	3.00 ± 0.57	60.06 ± 1.11***	314.20
Diazepam (2)	2.00 ± 0.64	96.00 ± 1.98***	562.06

n=6. Data were analysed by one-way ANOVA followed by Turkey-Kramer Comparison test. ***** *$p<0.001$ compared to vehicle control.*

Effect on locomotor activity

The LSAF at dose of 50, 150 and 250 mg/kg produced 28.25, 40.04 and 57.43% reduction respectively in locomotor activity as compared with control animals receiving only the vehicle. However, the diazepam treated group revealed 90.90% decrease in locomotor activity compared to the control group.

Table 4. Effect of *Lepidium sativum* alkaloidal fraction on locomotor activity of mice

Dose (mg/kg, i.p.)	Basal Locomotor scoring (M ± SEM)	Locomotor scoring after 30 min (M ± SEM)	% decrease in locomotor activity
Vehicle	426 ± 3.92	432 ± 10.72	-
LSAF (50)	453 ± 9.84	325 ± 14.79***	28.25
LSAF (150)	507 ± 5.50	304 ± 12.89***	40.04
LSAF (250)	516 ± 7.57	218 ± 16.89***	57.43
Diazepam [2]	407 ± 4.93	37 ± 8.18***	90.90

n=6. Data were analysed by one-way ANOVA followed by Turkey-Kramer Comparison test. ***** *p< 0.001 compared to vehicle control.*

Effect on motor coordination

The LSAF at dose of 50, 150 and 250 mg/kg reduced the motor coordination in moving bars of rotarod. The maximum decrease in motor coordination (fall off time) was 52.29% at dose of 250 mg/kg whereas standard reference drug diazepam showed 76.06% decrease in fall off time.

Table 5. Effect of *Lepidium sativum* alkaloidal fraction on Locomotor coordination (Rota rod) of mice

Dose (mg/kg, i.p.)	Fall off time in sec		% Decrease in fall off time
	Basal	After drug treatment	
Vehicle	29.28	31.32 ± 3.78 ^{ns}	-
LSAF (50)	28.47	16.52 ± 4.58*	41.97
LSAF (150)	26.56	14.24 ± 3.50**	46.38
LSAF (250)	24.15	11.52 ± 2.74**	52.29
Diazepam (2)	31.50	7.54 ± 3.71***	76.06

n=6. Data were analysed by one-way ANOVA followed by Turkey-Kramer Comparison test.
*ns - non-significant, * p<0.05, **p<0.01 and ***p<0.001 compared to vehicle control.*

Elevated plus maze test

The LSAF treated animals at 50, 150 and 250 mg/kg doses showed increased first entry preference to the open arm. LSAF treatment dose dependently showed high to extremely significant (p<0.01-0.001) increase in duration of total time spent in open arm.

Table 6. Effect of *Lepidium sativum* alkaloidal fraction on anxiety induced using elevated plus maze-apparatus on mice.

Dose (mg/kg, i.p.)	% Preference to open arm	Time spent in min (M ± SEM)	
		Open arm	Enclosed arm
Vehicle	33.33	36.42 ± 6.11	263.58 ± 17.63
LSAF (50)	50.00	97.74 ± 12.40**	203.24 ± 16.44
LSAF (150)	66.66	109.20 ± 14.46**	194.35 ± 15.33
LSAF (250)	66.66	110.15 ± 10.28***	190.53 ± 16.46
Diazepam (2)	83.33	128.54 ± 11.89***	171.46 ± 14.47

n=6. Data were analysed by one-way ANOVA followed by Turkey-Kramer Comparison test.
***p<0.01, ***p<0.001 compared to vehicle control.*

Caudal immersion in hot water

The LSAF at doses of 50, 150 and 250 mg/kg respectively produced 46.66%, 63.12% and 82.08% increase in reaction time compared to control animals. The morphine treated group showed 96.29 % increase in reaction time.

Table: 7. Analgesic activity of *Lepidium sativum* alkaloidal fraction on rats (Tail immersion in hot water)

Dose (mg/kg, i.p.)	Reaction time in sec (M ± SEM)		% Increase in reaction time
	Basal reaction time	After 30min of drug treatment	
Vehicle	2.41 ± 0.54	2.51 ± 0.02	-
LSAF (50)	3.0 ± 0.68	4.4 ± 0.08***	46.66
LSAF (150)	2.44 ± 0.47	3.98 ± 0.07***	63.12
LSAF (250)	2.40 ± 0.37	4.37 ± 0.05***	82.08
Morphine(5)	2.43 ± 0.47	4.77 ± 0.06***	96.29

n=6. Data were analysed by one-way ANOVA followed by Turkey-Kramer Comparison test.
****p<0.001 compared to vehicle control.*

Acetic acid induced writhing

The LSAF at 250 mg/kg dose showed extremely significant analgesic response ($p<0.001$) against acetic acid induced chemical stimulant with 64.32% decrease in number of wriths. The morphine treated group showed 89.18% protection in number of wriths compared to vehicle control group.

Table 8. Analgesic activity of *Lepidium sativum* alkaloidal fraction on rats (Acetic acid induced writhing)

Dose [mg/kg, i.p.]	Writhing in 10 min [M ± SEM]	% Protection
Vehicle	18.5 ± 0.05	-
LSAF [50]	14.5 ± 0.84*	21.62
LSAF [150]	13.0 ± 1.19**	29.72
LSAF [250]	6.6 ± 1.20***	64.32
Morphine [5]	2.0 ± 0.31***	89.18

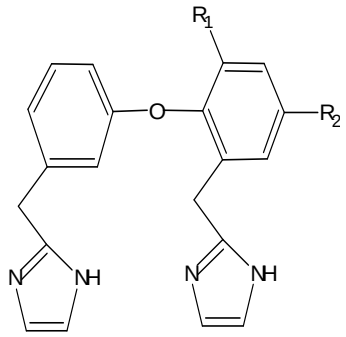
n=6. Data were analysed by one-way ANOVA followed by Turkey-Kramer Comparison test.
**p<0.05, **p<0.01, ***p<0.001 compared to vehicle control.*

This study compiles the effect of LSAF on several neuropharmacological and behavioral animal models e.g. thiopental-induced hypnosis, locomotor activity, elevated plus maze and tail immersion test to investigate the possible central effect. These tests are classical models for screening CNS action providing information on depressant or stimulant, psychomotor performance, anxiolytic, myorelaxant and analgesic activity profile.

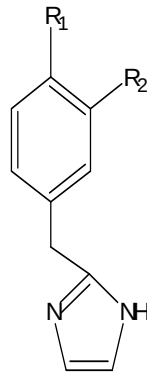
Alkaloid content was found to be 0.29% in the seed variety investigated here. The major components of this alkaloid fraction are lepidine and semilepidine, a rare group of imidazole alkaloid.

The active moiety in this alkaloid is 2- benzyl Imidazole or imidazoline. Intraperitoneal acute toxicity study to determine median lethal dose [LD_{50}] in mice suggests relatively low toxicity of *L. sativum* alkaloid. LD_{50} was found to be 2204.95 mg/kg [i.p.]. The causes of motility observed in very high doses are suggestively increased heart rate, respiratory depression, cramps in

abdomen and change in body posture as evidenced from the 4 h close extensive observation and 72 h intermittent observations indicating profound effect on CNS.



	R_1	R_2
Lepidine A	OCH_3	H
Lepidine B	OH	H
Lepidine C	H	OCH_3



	R_1	R_2
Semilepidine	OH	H
Semilepidine	OH	OCH_3

In the present study, the central effect of *L. sativum* total alkaloid has been evaluated at 50, 150 and 250 mg/kg [i.p.] doses. The results of general pharmacological observation indicated that the total alkaloid of *L. sativum* has influenced the general behavioral profile, as evidenced by decrease in the alertness, reactivity to touch and auditory stimuli indicative of depressant profile. *L. sativum* alkaloidal fraction produced a significant increase in thiopental sodium induced hypnotic effect in a dose dependent manner, thus suggesting a profound depressant activity on the CNS. The observed sedative effect may be related to an interaction with benzodiazepines and related compounds that bind to GABA receptors in the CNS.

Locomotor activity is considered as an index of alertness and a decrease in it is indicative of sedative activity [24]. *L. sativum* total alkaloid decreased locomotor activity at all the tested doses reinforcing the CNS depressant effect. The alcoholic tincture of *L. sativum* is reported to possess sedative and anticonvulsant activity [25].

Significant myorelaxant effect was observed in 250 mg/kg dose of LSAF with decrease in latency of fall offs and total time spent on the rotarod bars. Alkaloidal fraction showed lack of motor coordination and muscle relaxant activity in mice.

The elevated plus maze is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli [fear of a novel open space and fear of balancing on a relatively narrow, raised platform] that can induce anxiety in humans. An anxiolytic agent increases the time spent in open arms and decreases the time spent in enclosed arm of the elevated plus maze. In the present study, intraperitoneal administration of LSAF demonstrated an anxiolytic like effect in mice, as it significantly increased the total time spent in open arms and percent of first entry preference to open arms [26].

The standard reference drug diazepam [benzodiazepine] act as a anxiolytic at low doses and also produce sedation and myorelaxant effect at higher doses [27]. The possible depressant, anxiolytic and myorelaxant mechanism of action of LSAF could be due to the binding with $GABA_A$-benzodiazepine receptor complex.

Acetic acid induced abdominal constriction test is usually used for the evaluation of peripheral analgesic activity. The abdominal writhing elicited by acetic acid has been reported to be a model of visceral pain very sensitive and less selective model [central and/ or peripheral] that enables the detection of antinoiciceptive activity of compounds in laboratory animals. The i.p. injection of acetic acid irritates visceral surface of intestine leading to release of bradykinin, histamine and prostaglandin. Collier et al, 1968 [28] proposed that acetic acid acts indirectly by releasing endogenous inflammatory mediators [mainly prostacyclin and prostaglandin- E], which stimulate neurons [A-δ fibres] in pain pathway. Stimulation of A-δ fibres causes a sensation of sharp well localized pain. Any agent that lower the number of writhes have preferably a peripheral mechanism of pain inhibition, but A-δ fibres are also sensitive to narcotics and other centrally acting agents acting by inhibition of neurotransmitters [e.g. serotonin, noradrenaline, glutamine and substance- P] in algesic pathway [29]. *L. sativum* alkaloid showed analgesic activity in acetic acid induced writhing test indicating preferably peripherally mediating analgesic activity. However, this model may not be able to indicate clearly the mechanism of analgesic effect of alkaloid fraction because other agents such as antihistamines and myorelaxants are also able to reduce the pain induced by acetic acid [30].

Tail immersion method is used to evaluate central mechanism of analgesic activity. In this protocol painful reactions in animals are produced by thermal stimulus, by dipping the tip of tail in hot water. Thermal stimulation in tail immersion test is a supraspinally mediated pain response and any agent prolonging the response supposing acts centrally. LSAF at 250 mg/kg dose showed 64.32% decrease in tail withdrawal response.

Analgesic effect against thermal noxious stimuli may be elicited through opoid receptors or through modulation of several neurotransmitters like serotonin, noradrenaline and substance-P involved in algesia pathway. It is also reported that inhibition of pain could arise not only from the presence of opioids and/ or opoidiomimetics but could also arise from the presence of phenolics, steroidal or alkaloidal constituents [31-32]. Drugs interacting with opioid receptors inhibit both peripheral as well as centrally induced pain and algesic response [33].

Imidazoles are an important class of heterocycles including many substances of both biological and chemical interest. They are part of a large number of highly significant biomolecules such as the essential amino acids and related compounds, biotin and the imidazole alkaloids. Imidazole drugs have broad applications in many clinical therapies like antibacterial, antifungal, anti-inflammatory, histamine agonist etc. [34]. Behavioral pharmacology of imidazole is reported to be depressant of motor activity in mice and rats [35]. Imidazole 4- acetic acid is a naturally occurring metabolite in brain, suggestively an oxidation product of histamine. Imidazole 4-acetic acid displays definite partial agonist characteristic as an enhancer of benzodiazepine binding to the $GABA_A$ receptor complex. It also binds with imidazoline 1_1 receptor. Owing to these properties i.p. administration of imidazole 4- acetic acid in experimental animals leads to sleep-

like state [36]. Analgesic activity is reported in some 2- substituted and 1, 2-disubstituted imidazole derivatives [37].

The majority of noradrenaline containing neurons in the CNS has their cell bodies in the nucleus locus coeruleus, and is thought to be involved with number of physiological properties such as sleep and awake, and response to noxious stimuli [38-40].

Structurally similar compounds of lepidine having imidazoline back bone e.g., tolazoline [[2-benzyl]-4, 5-dihydro-1H imidazole] act as a peripheral α-blocker inducing vasodilation [41]. Dexmedetomidine [4-{1-[2, 3-dimethyl phenyl] ethyl}-3-H-imidazole] and clonidine [2, 6-dichlorophenyl-4, 5-dihydro-imidazolidine] act as a central sympatholytic agent inducing partial agonist activity at α_{2A}-receptors in brain stem and medulla. Dexmedetomidine has sedative, analgesic and anxiolytic effects and is used as a sedative medication in intensive care unit as it is free of respiratory depression [42].

Clonidine acts as an agonist in presynaptic central α_{2A}-receptors via imidazoline 1_1 receptors resulting in suppression of efferent sympathetic pathway, subsequently decrease in blood pressure and vascular tone. Opioid and α_2-adrenergic systems converge on the same effectors in many functional systems of brain. Clonidine is useful as morphine substitute in postoperative analgesia, in severe cancer pain refractory to opiate, opiate and nicotine withdrawal and in diabetic neuropathic pain [41].

The structure of dimeric lepidine and semilepidine shows substitution in 2, 3, 4 and 5 positions of the phenyl ring. It is reported that 2, 6-disubstituted compounds are the most active as pre-junctional α_2-agonist but the activity can be maintained in compounds with substitution in other positions on the phenyl ring irrespective of imidazolidine or imidazoline nucleus. For potent central α-adrenoceptor action the phenyl and imidazoline rings should be aplanar with 2, 3 or 2, 5-disubstitutions. Substitution in 3, 4 or 5- positions of phenyl ring preclude potent activity at H_2- receptor sites while maintaining α -receptor activity [43].

The observed CNS depressant activity of *L. sativum* alkaloid [lepidine and semilepidine] may be due to interaction with central presynaptic α_{2A}-receptor as partial agonist by decreasing noradrenergic drive resulting in CNS depression, as decrease in central sympathetic drive is related to depression [44]. This mechanism may also be involved in inducing potent analgesic activity sharing the effectors of opioid system. Sedative response is also a part of it. Sedative, antianxiety and myorelaxant response may also involve enhancement of benzodiazepine binding to $GABA_A$ receptor complex.

CONCLUSION

The results of the present study demonstrate sedative, anxiolytic, myorelaxant and analgesic activity of *L. sativum* alkaloid belongs to imidazole category with poly substituted phenyl-imidazoline basic nucleus. Imidazole, imidazolines and imidazolidines are potent centrally acting molecules interacting with α_{2A}-adrenoreceptors, imidazoline1_1-receptor and GABA receptor. All the observed activities are in accordance to responses mediated by these receptors. The exact mechanism of action for interaction with central adrenergic transmission, opioid receptors and

$GABA_A$-receptor complex remains to be elucidated. Further studies on analgesic and CNS depressant profile of the alkaloid is still going on in our laboratory to explore exact mode of action on specific interaction with neurotransmitters like noradrenaline, GABA and opioid ligands.

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