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Evaluation of antianxiety activity of Linalyl acetate in Swiss albino Mice

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Abstract

Context: Linalyl acetate is major chemical constituent of lavender (*Lavandula angustifolia*, family-Lamiaceae), the plant extract shows anxiolytic effect in aromatherapy so there are possibility of linalyl acetate have anxiolytic effect. Already known effect of linalyl acetate are anti-inflammatory, analgesic and antihypertensive. Despite a long tradition of use, there was no evidence that phytochemical linalyl alone is responsible for activity. Hence work has been carried out to specify phytochemical linalyl acetate is responsible for anxiolytic effect of lavender.

Objective: Three doses of linalyl aceatae were subjected to dertermine anxiolytic activity on evidence of anxiolytic use of plant extract and to determine the most potent dose of linalyl acetate.

Materials and methods: All doses of Linalyl acetate were evaluated for anxiolytic effect in mice by using elevated plus maze, light and dark apparatus. And for locomotor activity open field apparatus and actophotometer were used. Alprazolam was used as the standard drug.

Results: Amongest all doses, linalyl acetate (400 mg/kg) dose was found most potent.

Conclusion: The current study demonstrates statistically significant dose-dependent antianxiety activity of linalyl acetate.

Keywords: Antianxiety effect, Lanvender, Elevated plus maze, light and dark apparatus, open field apparatus, actophotometer.

INTRODUCTION

Anxiety is body's natural response to stress and it is defined as a state of mental illness, excessive fear associated with over excitation of sympathetic system, worry, nervousness and carefulness syndrome which may result various CNS disorders if not treated (Ninan et al., 2001). This is mainly due to the unidentified symptoms of anxiety hence not get proper treatment (Pasquini et al., 2009). Physician mistake in differentiating anxiety and physical illness hence only 23% patients receive appropriate treatment (Roy et al., 2004). Anxious individuals are more prone to develop other vital organ complications than normal individuals (Kessler et al., 2010).

Anxiolytic plants also evaluated for their antioxidant activities (Amin et al., 2017). The elevated

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plus maze has been validated for the evaluation of anxiolytic effect of bioactive components of plants as well as for synthetic pharmaceutical agents (Walf et al., 2007). Humans on EPM shows similar behaviour as observed in rodents (Biedermann et al., 2017).

Crawley developed extrapolation experiment in the light and dark apparatus (Crawley et al., 1980). Various neurotransmitters in brain glutamate, GABA, sympathomimetic agent and catecholamines are associated with anxious state by different mechanism (Nutt et al., 2005). The oxidative stress in brain results multiple neuropsychiatric disorders such as anxiety and depression (Bouayed et al., 2009).

Linalyl acetate is an monoterpene and carboxylate ester, it is present as major bioactive component in essential oil extract of many plants for example bergamot and lavender. It is colorless liquid which is insoluble in water. I choose linalyl acetate for evaluation of its anxiolytic activity as it is major chemical constituent of lavender essential oil (Verma et al., 2010) .The essential oil extract of lavender plant consists two major chemicals 36.8% of linalool and 34.2% of linalyl acetate (Schuwald et al., 2013).

Anxiety Models

Elevated Plus Maze- Anxiety related behaviours are evaluated by EPM by following Lister's method. The % of duration spent in open arms of maze was calculated as the total duration spent in open arm of maze/total time of exposure on maze. The % total numbers of entrances in open area of EPM were calculated as total transitions in open part of maze/number of transitions in both open and closed arms. The increase total exposure period in open area of EPM is observed as indicator of anxiolytic potential of experimental substance and motor activity indicator is total approach to closed arm of maze in test (Biedermann et al., 2017). Other evidences of anxiety disorder observed in EPM testing are head dipping (sticking of head to the floor of maze), stretched posture, seizures, grooming (cleaning of mouth by paws), (Padurariu and Lulia et al., 2017).

Light and dark model - The LD model has two compartments and consists of a box (40 x 25 x 25 cm) divided into two compartment, one light compartment and another dark compartment are connected to each other and have an opening (4 x 4 cm) between them (Crawley et al., 1980). The duration of exposure in the darkened or light portions of the LD box was measured (Kalonia and Kumar et al., 2007).

Open field- Open field apparatus is simple test apparatus for experimental animals generally performed for locomotors activity testing in rodents. The parameters recorded in the OPM are the

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frequency of crossing square, total numbers of squares crossed (cross of square counts by entry as well exit of four paws of rodent in that), center square entries, defecation and urination frequency (Seibenhener et al., 2015).

Actophotometer- The measure of locomotors activity in rodents done by actophotometer, it gives an accurate measure of locomotors activity. The principle of actophotometer based on the total number of photocell beam crossed by rodent during testing session (Kalonia and Kumar, 2007).

Linalyl acetate

IUPAC name: 3, 7-dimethyl-1, 6-octadien-3-yl acetate, Chemical formula: $C_{12}H_{20}O_2$ or $CH_3COOC_{10}H_{17}$; Linalyl acetate is an monoterpene and carboxylated ester, it is the main component of lavender (Verma et al., 2009). Linalyl acetate is not soluble in water but its solubility in tween 80's solution and diethyl ether and it is a colourless liquid and sweet in taste. Monoterpenes linalool and linalyl acetate present in oil of lavender are responsible for its anxiolytic activity (Linck et al., 2010; Lopez et al., 2017). Previous reported activity of linalyl acetate antibacterial activity (Trombetta et al., 2005), anti-inflammatory (Peana et al., 2002), local anesthetic (Ghelardini et al., 1999) and decrease blood pressure (Kim R J et al., 2017)

MATERIAL AND METHODS

Experimental Subjects

Swiss albino female mice (weight 25-35 g) have been purchased from LUVAS' animal house, Hisar (Haryana, India). Rodents were procured to the Central Animal House of MDU, Rohtak after aproval of Institutional Animal Ethics Committee (IAEC) in a meeting held at Maharshi Dayanand University, Rohtak, Haryana, India. They were corroborated under standard conditions for two weeks. After that mice were divided randomly into 5 groups (n=5). The handling of animal has been taken as per guidelines of CPCSEA, Dept. of Animal Husbandry and Dairying Ministry of Agriculture and Farmers Welfare, Government of India.

Selection of doses

Alprazolam was used as standard in the present study and normal saline solution used as vehicle. All drugs are dissolved in water for administration (Attia et al., 2014; Kumar et al., 2013; Mendaros et al., 2018). Linalyl acetate has been suspended in 1% (v/v) polysorbate tween 80's solution in water solution and delivered per oral according to body weight, 3 different dose of linalyl acetate 200 mg/kg, 400 mg/kg and 800 mg/kg were administered for finding of most potent dose of Linalyl

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acetate. All sets of mice were treated with selected doses once daily for 15 days.

Experimental groups

Groups/sets (n=5) that were used for anxiolytic activity:

For most potent dose estimation of linalyl acetate:

Set 1: Control

Set 2: Alprazolam (1 mg/kg p.o.)

Set 3-5: Linalyl acetate (200, 400 and 800 mg/kg respectively administered through oral route.

RESULT

Relative anxiolytic activity profiles of different doses (200,400 and 800mg/kg) of linalyl acetate, alprazolam and control are shown in table

1. Elevated plus maze test-

a) Number of accesses- Increase in number of accesses in C vs A show significant at p <0.01; C vs LA 200 p<0.01; C vs LA 400 show significant at p<0.001; C vs LA 800 show significant at p<0.01. A vs LA 200 do not show any significant; A vs LA 400 do not show any significant and A vs LA 800 do not show any significant. LA 200 vs LA 400 do not show any significant; LA 200 vs LA 800 do not show any significant. LA 400 vs LA 800 do not show any significant as represented in figure (1).

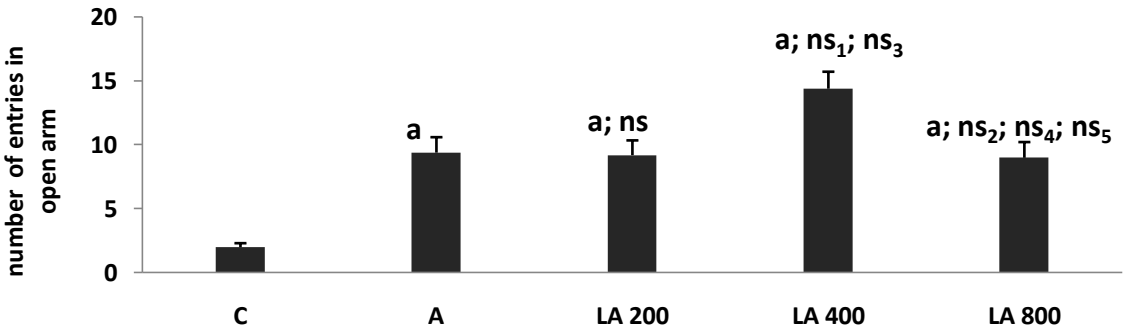

Figure.1.Various treatments' impact on number of open arm accesses in EPM in mice

N=5 in each set.

All values are manifested as mean ± S.E.M. One way ANOVA pursued by Tukey's test has been used for data exploration.

a: p<0.01 C vs A or C vs LA200 or C vs LA 400 or C vs LA 800, ns: A vs LA 200 not show significant; ns_1: A vs LA 400 non significant; ns_2: A vs LA 800 non significant; ns_3: LA 200 vs LA

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400 non significant; ns_4: LA 200 vs LA 800 non significant; ns_5: LA 400 vs LA 800 not show significant.

C: Control group; A: Alprazolam; LA 200: Linalyl acetate 200 mg/kg; LA 400: Linalyl acetate 400 mg/kg; LA 800: Linalyl acetate 800 mg/kg.

b) Time spent in open arm- Increase in total time spent in open arm in C vs A show significant at p<0.001; C vs LA 200 show significant at p <0.001; C vs LA 400 show significant at p<0.001; C vs LA 800 show significant at p<0.001. A vs LA 200 do not show any significant; A vs LA 400 show significant at p<0.05; A vs LA 800 do not show any significant. LA 200 vs LA 400 do not show any significant; LA 200 vs LA 800 do not show any significant. LA 400 vs LA 800 do not show any significant as represented in figure (2).

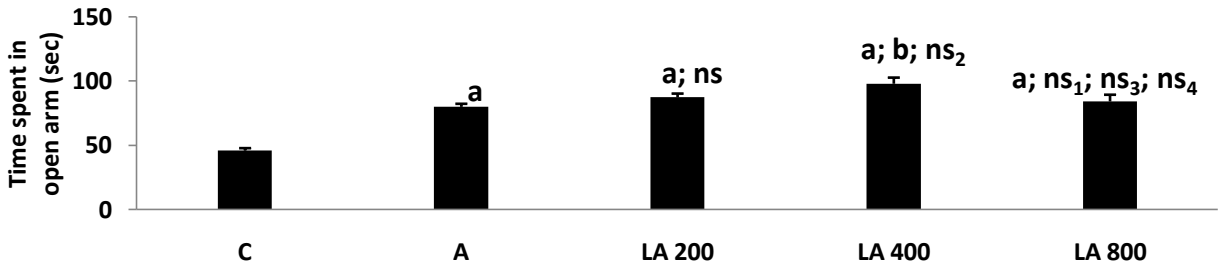

Figure2. Different treatments' effect on time spent in open arm of EPM in mice

N=5 in each set.

All values are manifested as mean ± S.E.M. Data were explored by one way ANOVA pursued by Tukey's test.

a: p<0.001 C vs A or C vs LA 200 or C vs LA 400 or C vs LA 800; b: p<0.05 A vs LA 400 A; ns: A vs LA 200 non significant; ns_1: A vs LA 800 non significant; ns_2: LA 200 vs LA 400 non significant; ns_3: LA 200 vs LA 800 non significant; ns_4: LA 400 vs LA 800 non significant.

C: Control group; A: Alprazolam; LA 200: Linalyl acetate 200 mg/kg; LA 400: Linalyl acetate 400 mg/kg; LA 800: Linalyl acetate 800 mg/kg.

2. Light and Dark Model-

a) Number of entries in light chamber - Increase in number of entrances in light compartment in C vs A show significant at p<0.01; C vs LA 200 show significant at p<0.001; C vs LA 400 show significant at p<0.001; C vs LA 800 show significant at p<0.05. A vs LA 200 do not show any significant; A vs LA 400 do not show any significant; A vs LA 800 do not show any significant. LA 200 vs LA 400 do not show any significant; LA 200 vs LA 800 do not show any significant. LA

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400 vs LA 800 do not show any significant as represented in figure (3).

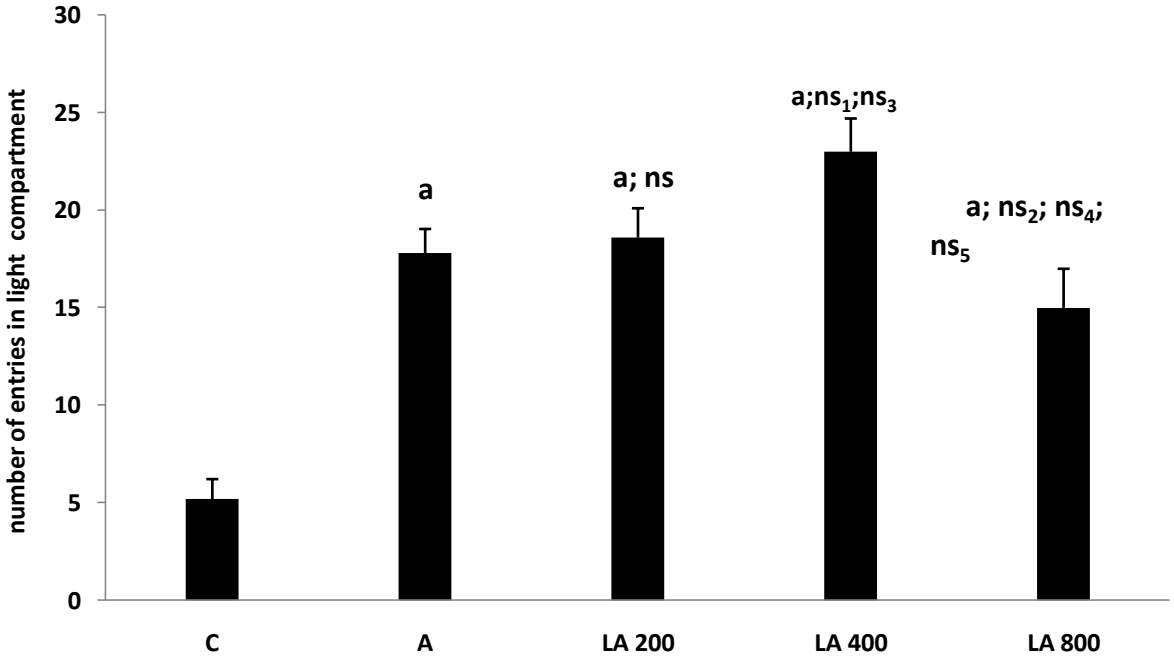

Figure3.Various treatments' effect on number of entries in light chamber in LD in mice

N= 5 in each set

Values are manifested as mean ± S.E.M. One way ANOVA pursued by Tukey's test has been used for data exploration.

a: $p<0.05$ C vs A or C vs LA 200 or C vs LA 400 or C vs LA 800; ns: A vs LA 200 non significant; ns_1: A vs LA 400 non significant; ns_2: A vs LA 800 non significant; ns_3: LA 200 vs LA 400 non significant; ns_4: LA 200 vs LA 800 non significant; ns_5: LA 400 vs LA 800 non significant.

C: Control group; A: Alprazolam; LA 200: Linalyl acetate 200 mg/kg; LA 400: Linalyl acetate 400 mg/kg; LA 800: Linalyl acetate 800 mg/kg.

b) Time spent in light chamber of LD- Increase in time spent in light compartment in C vs A show significant at $p<0.001$; C vs LA 200 show significant at $p<0.001$; C vs LA 400 show significant at $p<0.001$; C vs LA 800 show significant at $p<0.01$. A vs LA 200 do not show any significant; A vs LA 400 do not show any significant. LA 200 vs LA 400 show significant at $p<0.05$ as represented in figure (4).

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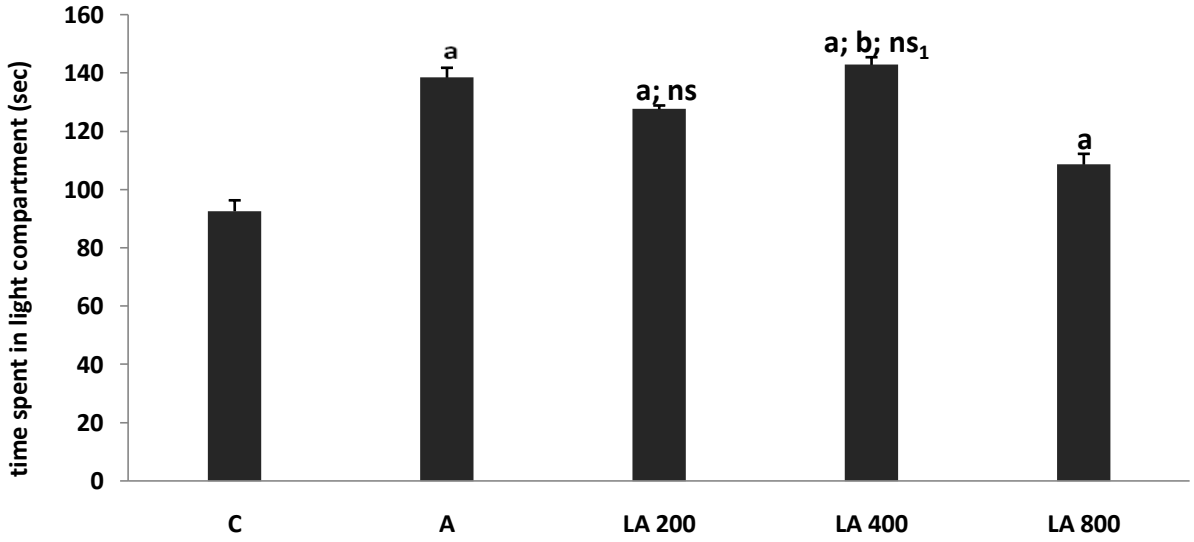

Figure4. Impact of various treatments on time spent in bright chamber of LD in mice

N= 5 in each set

Values are manifested as mean ± S.E.M. One way ANOVA persued by Tukey's test has been used for data exploration.

a: $p<0.01$ for C vs A or C vs LA 200 or C vs LA 400 or C vs LA 800; ns: A vs LA 200 non significant; ns_1: A vs LA 400 non significant; b: $p<0.05$ for LA200 vs LA 400.

C: Control group; A: Alprazolam; LA 200: Linalyl acetate 200 mg/kg; LA 400: Linalyl acetate 400 mg/kg; LA 800: Linalyl acetate 800 mg/kg.

3. Actophotometer

Number of beams crossed- Increase in number of beams crossed in C vs A show significant at $p<0.001$; C vs LA 200 show significant at $p<0.01$; C vs LA 400 show significant at $p<0.001$; C vs LA 800 show significant at $p<0.001$. A vs LA 200 show significant at $p<0.001$; A vs LA 400 show significant at $p<0.001$; A vs LA 800 show significant $p<0.001$. LA 200 vs LA 400 show significant at $p<0.001$; LA 200 vs LA 800 show significant at $p<0.001$. LA 400 vs LA 800 show significant at $p<0.001$ as represented in figure (5).

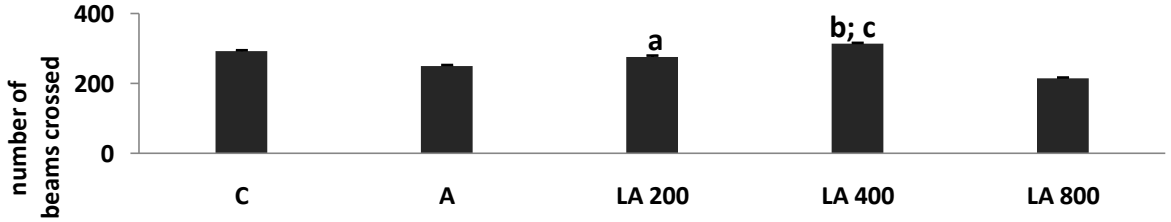

Figure5. Different treatments' impact on number of beams crossed in Actophotometer in mice

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N= 5 in each set

Values are manifested as mean ± S.E.M., n=5 in each set. One way ANOVA pursued by Tukey's test has been used for data exploration

a: $p<0.001$ for A vs LA 200; b: $p<0.001$ A vs LA 400; c: $p<0.001$ LA 200 vs LA 400.

C: Control group; A: Alprazolam; LA 200: Linalyl acetate 200 mg/kg; LA 400: Linalyl acetate 400 mg/kg; LA 800: Linalyl acetate 800 mg/kg.

4. Open field

a) Number of squares crossed other than center- Increase in number of squares crossing at periphery in C vs A show significant at $p<0.001$; C vs LA 200 show significant at $p<0.001$; C vs LA 400 show significant at $p<0.001$; C vs LA 800 show significant at $p<0.01$. A vs LA 200 do not show any significant; A vs LA 400 show significant at $p<0.05$; A vs LA 800 do not show any significant. LA 200 vs LA 400 do not show any significant; LA 200 vs LA 800 do not show any significant as represented in figure (6).

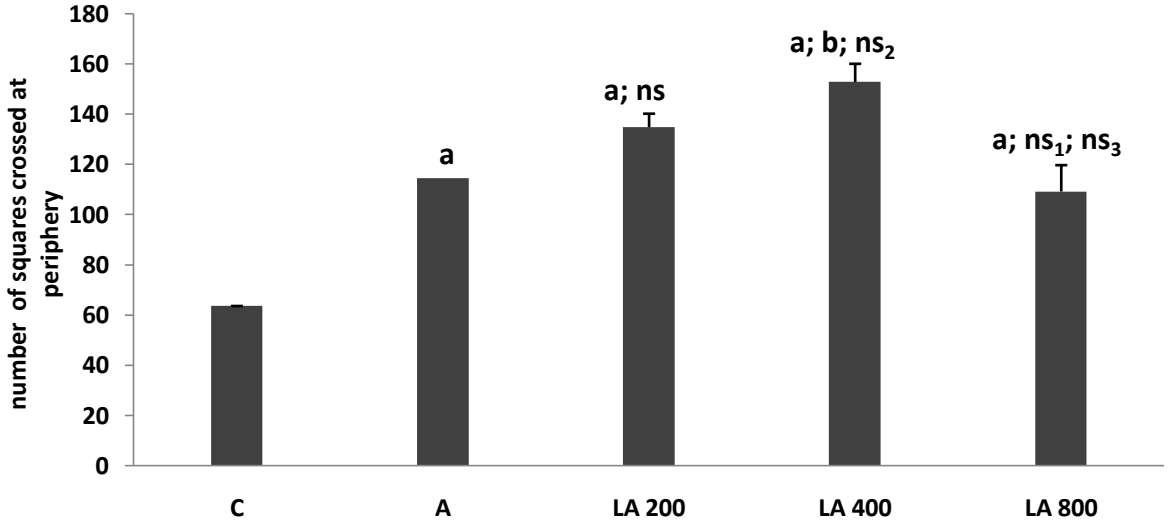

Figure6. Impact of different treatments on number of squares crossed at periphery in OPM in mice

N= 5 in each set

Values are manifested as mean ± S.E.M. One way ANOVA pursued by Tukey's test has been used for data exploration.

a: $p<0.01$ for C vs A or C vs LA 200 or C vs LA 400 or C vs LA 800; b: $p<0.05$ for A vs LA 400; ns: A vs LA 200 non significant; ns_1: A vs LA 800 non significant; ns_2: LA 200 vs LA 400 non significant; ns_3: LA 200 vs LA 800.

C: Control group; A: Alprazolam; LA 200: Linalyl acetate 200 mg/kg; LA 400: Linalyl acetate 400

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mg/kg; LA 800: Linalyl acetate 800 mg/kg.

b) Number of squares crossed at center- Increase in numbers of squares cross at center of open field in C vs A show significant at p<0.01; C vs LA 200 show significant at p<0.001; C vs LA 400 show significant at p<0.001; C vs LA 800 show significant at p<0.05. A vs LA 200 do not show any significant; A vs LA 400 do not show any significant; A vs LA 800 do not show any significant. LA 200 vs LA 400 do not show any significant; LA 200 vs LA 800 do not show any significant. LA 400 vs LA 800 do not show any significant as represented in figure (7).

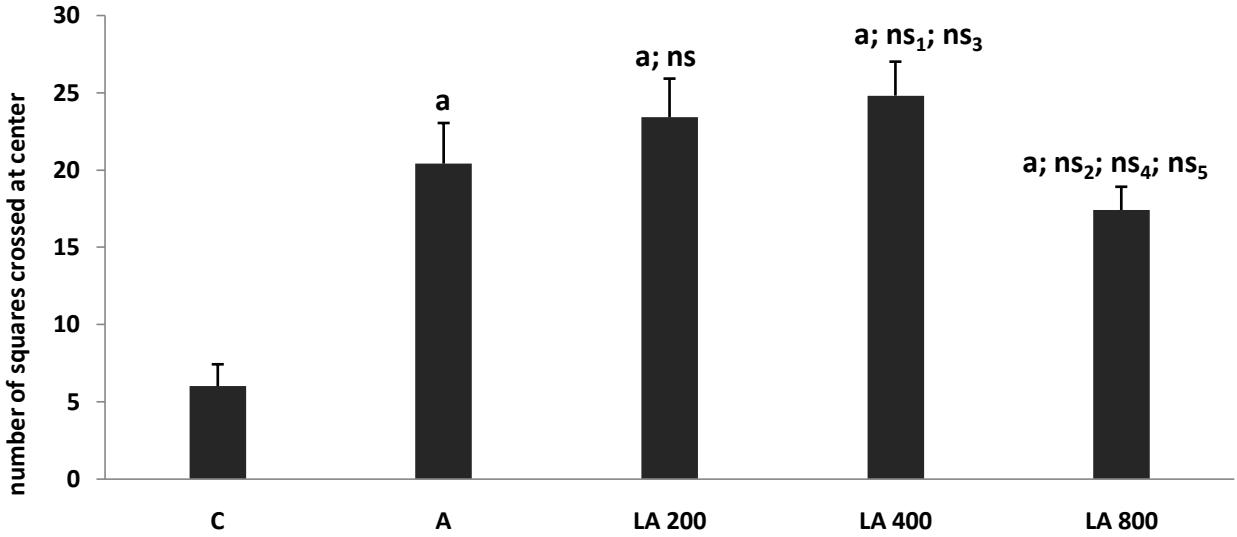

Figure7. Influence of different treatments on numbers of squares cross at center in OPM in mice

N= 5 in each set

Values are manifested as mean ± S.E.M. One way ANOVA pursued by Tukey's test has been used for data exploration.

a: p<0.05 for C vs A or C vs LA 200 or C vs LA 400 or C vs LA 800; ns: A vs LA 200 non significant; ns_1: A vs LA 400 non significant; ns_2: A vs LA 800 non significant; ns_3: LA 200 vs LA 400 non significant; ns_4: LA 200 vs LA 800 non significant; ns_5: LA 400 vs LA 800 non significant. C: Control group; A: Alprazolam; LA 200: Linalyl acetate 200 mg/kg; LA 400: Linalyl acetate 400 mg/kg; LA 800: Linalyl acetate 800 mg/kg

DISCUSSION

In the present study, different dose of linalyl acetate (200, 400 and 800 mg/kg) was used for evaluation of anxiety like behaviour in mice. These different doses were administered for 15 successive days. On day 15th, after 15 min of last administration, anxiolytic test were performed on

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EPM and LD and after 15 min of anxiolytic test behavioural parameters were performed on open field apparatus and actophotometer. Treated with linalyl acetate (200 mg/kg) for 15 consecutive days considerably produced anti-anxiety effect as expressed by an insignificant elevation in entrances and total exposure period to open arm of EPM with respect to standard drug (alprazolam). In the elevated plus maze, avoidance of open arms and preference to closed arm indicates state of anxiety like disorder this is supported by literature (Pellow et al., 1985). In EPM, lesser duration of exposure in open arm and less number of transitions in unclosed arms are the indicators of anxiety in rodents (Kumar et al., 2013). The antianxiety potential of plant extracts was evaluated by anxiolytic models EPM and LD (Bourin et al., 2015). In previous study, anxiolytic activity of *C. sativum* has been evaluated by light and dark model by using diazepam as standard drug (Mahendra et al., 2011).

After 15 min of light and dark model test, behavioural test was performed in actophotometer. The treated group expressed a meaningful elevation in number of beams crossed in actophotometer test as compared to alprazolam (Kaur et al., 2017). Locomotors activity of combination of morphine with fluoxetine decrease as compared to control group was reported in previous study, this reduction was due to sedative effect of combination treatment (Votava et al., 2001).

In open field maze behavioural test was performed after 15 min of actophotmeter testing. The test expressed elevation in total number of squares covered near walls and elevation in number of squares crossed at cretic in open field maze as compared to alprazolam. This indicated linalyl acetate (200 mg/kg) increase locomotors activities along with its anxiolytic effect (Sestakoba et al., 2013; Jaiswal et al., 2002).

Linalyl acetate (400 mg/kg) administered for 15 succeeding days significantly represented anti-anxiety effect that indicated by an insignificant rise in total number entrances and a meaningful elevation in exposure duration in open arm in EPM test, an insignificant rise in number of moves in light compartment and an insignificant elevation in duration in bright chamber in LD test as compared to alprazolam. The anxiolytic effect of linalyl acetate also demonstrated by a meaningful elevation in number of beams crossed in actophotometer test, a meaningful rise in number of squares covered at near walls and an insignificant elevation in total number of squares covered at centre in open field maze as compared to alprazolam.

After 15 days successive administration of linalyl acetate (800 mg/kg) produced antianxiety effect which indicated by a insignificant elevation in total number of transitions in open arm and insignificant elevation in total duration of exposure in open arm of EPM, an insignificant rise in number of entries in bright compartment and an insignificant elevation in duration of

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exposure in bright chamber in LD test as compared to alprazolam. Linalyl acetate treated group expressed a meaningful reduction in total number of beams crossed in actophotometer test, an insignificant increase in numbers of crossings of squares at other than cretic and an insignificant increase in total number of squares covered at centre in open field apparatus test as compared to alprazolam. Hence, linalyl acetate (800 mg/kg) anxiolytic activity is comparatively less than alprazolam. Linalyl acetate (800 mg/kg) treated group expressed a significantly decline number of beams crossed in actophotometer as compare to linalyl acetate (400 mg/kg) treated mice which indicates that the high doses of linalyl acetate have sedative effect. This is supported by previous studies as monoterpenes present in lavender oil mainly at high concentration linalool increase noradrenaline level in brain and rise in NE increase contraction of smooth muscles by different processes (Poyton et al., 2015). In the present study, decrease in number of beams crossed in actophotometer by alprazolam treated group demonstrates the decrease in locomotor activity due to sedative effect of alprazolam and this is also supported by previous study. But significant increase in number of beams crossed in actophotometer in linalyl acetate (400 mg/kg) treatment group indicates no sedative effect of linalyl acetate at this dose.

In present study, from above data exploration (400 mg/kg) dose of linalyl acetate was found as most potent dose.

Conclusion

In animal models of anxiety linalyl acetate (200, 400 and 800 mg/kg) produced antianxiety effect. But, the linalyl acetate 800 mg/kg doses have sedative effect comparatively equal or high than alprazolam along with anxiolytic potential. It indicates that the increase in dose of linalyl acetate showed no further increase in anxiolytic potential and expressed increase in sedative effect. But linalyl acetate 400 mg/kg dose did not expressed decrease in locomotors activities, it means linalyl acetate not expressed sedative effect at low dose. And linalyl acetate 400 mg/kg was recorded as most potent dose for its anxiolytic action.

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