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EVALUATION OF ANXIOLYTIC ACTIVITY OF HOMEOPATHIC REMEDY *ARGENTUM NITRICUM* OF 30 C POTENCY AFTER CHRONIC ADMINISTRATION IN RODENTS

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ABSTRACT: Anxiety affects a large number of the population that creates an economic burden. Various traditional medicines like barbiturates, benzodiazepines and SSRI's are used as anxiolytics but due to their potential to produce adverse effect on cognition limits their chronic use. Several homeopathic remedies are used for treating anxiety. For the first time this study was designed to determine anxiolytic activity of 30C *Argentum nitricum*. The remedy was compared with escitalopram. For this purpose several behavioral parameters were analyzed using head dip test, home cage activity, open field test and light and dark test. Distilled water, *Argentum nitricum* and escitalopram were administered orally to three groups of animals. The effects were recorded after 30th and 60th days of dosing. The results were compared statistically using one way ANOVA and Tuckey test was also being done for the multiple comparisons. The remedy highly significantly decreased the head dips and cage crossings after the 60th day of dosing ($p \leq 0.001$). The central squares crossings in the open field apparatus was increased significantly after 30th days of dosing, which was highly significantly decreased after the 60th day of dosing when compared with day 30th ($p \leq 0.001$). The percent time spent in light compartment it was found significantly increased in comparison with control ($p \leq 0.001$) after 60th days of dosing. In conclusion, we found that *Argentum nitricum* possess anxiolytic profile, but the use of low doses and high dilutions is advocated to avoid any toxicity.

INTRODUCTION: Anxiety is the anticipation of fear about the future. It is the feeling of apprehensions and uneasiness. People suffering from anxiety if not treated may suffer from secondary illness.

Several anxiolytics are used for the treatment of anxiety for example barbiturates, benzodiazepines, SSRI's etc. These drugs possess drastic adverse effects on cognition and memory and chronic use they may compromise their effectiveness.

Researchers are continually searching for alternative medicines with least adverse effect due to the above explained reasons. Several herbs are under investigation and are used as anxiolytics for example, *Corianderum sativum*, *Foeniculum vulgareae*, *Valeriana officinalis* etc.

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Various homeopathic remedies were found to be useful for curing state of anxiety, phobia, and emotional instability¹ for example *Calcarea carbonica* is recommended for person having fear of losing control, Kali phosphoricum for hypersensitive and impatient person, *Gelsimum sempervirensin* persons suffering from anxiety and don't want to face people². This study was designed to evaluate the effect of homeopathic remedy *Argentum nitricum* on neurobehavioral parameters. The source of *Argentum nitricum* is of mineral origin. Because of lack of scientific data the anxiolytic property of *Argentum nitricum* is not are prescribing it for same purpose. *Argentum nitricum* which is used for the treatment of anxiety (due to fear of height), for generalized anxiety disorder (GAD), nervousness, fear of open places and other conditions.

It is recommended for treating giddiness with shaking, worries, neuralgic pain which is relieved after emesis. It also acts as antiepileptic. It is the best remedy for treating persistent unilateral headache. This remedy is recommended in those who are hasty in nature. Such patients try to escape from the situation that creates anxiety in them and have fear of darkness and before any event they may suffer from diarrhea.

MATERIAL AND METHODS:

Animal Selection and Housing: Administrations of test and standard drugs were done in two species *i.e.* albino rats and mice for two months. The rats and mice selected had an average weight ranges from 0.15 to 0.18 kg and 0.025 - 0.030 kg respectively. Both species were arbitrarily ascribed into three groups, respectively, each containing ten animals, one group of animals served as a control group, another group of animals served as treated group for standard drug escitalopram while the third group served as treated group for *Argentum nitricum* for CNS activities. Animals were kept at 20 to 23 °C room temperature with 12 / 12 h light / dark cycle at the Department of Pharmacology, University of Karachi and animals had easy access to water and food *ad libitum*. Two animals were kept per cage at least 1 week before start of dosing.

Dosing: 30C potency of *Argentum nitricum* was selected on the basis of literature survey which proved that 30C potency is a medium potency dose

and had shown effects in the treatment of chronic disease³.

Dose Calculation: 2 drops of 30C potency in 30 ml, then take 1tsp out of this dilution, is an adult dose. For 0.025 kg mice the dose will be = 0.01 ml or 10 micro liter. For 0.15 kg rats the dose will be = 0.066 ml or 60 µl.

Distilled water was administered orally to control group, while *Argentum nitricum* and Escitalopram were also been given orally to the animals for the determination of their effect on CNS activities. On day 30th and day 60th results were observed. The Helsinki Resolution 1964 specifications for animal handling were followed for behavioral analysis. BASR of University of Karachi had approved this study, Vide Resolution no.10 (50).

General Behavioral Activities:

Hole Poking Test: For this test, a hole poking box which is made of wood having 3 holes per side was used. For evaluation of activity all groups of animals were placed individually in the box for 10 minutes and hole poking were counted. The test was done after 30th and 60th day of dosing⁴. Animals after facing a novel atmosphere will explore⁵. The animal may explore or avoid novelty⁶.

Home Cage Crossing Test: The apparatus is made of clear plexiglass cages with transparent floor. The exploratory activity was monitored. The rats were placed individually in these cages⁷. At 10 min the numbers of cage crossings were noted on 30th day and 60th day of dosing for 10 min.

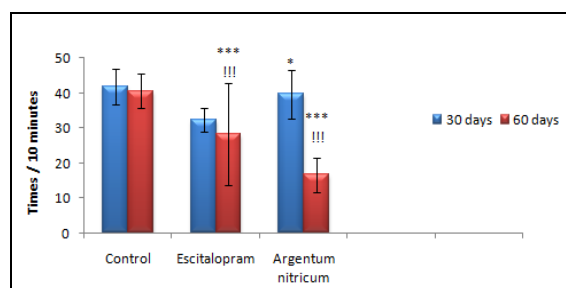
Open Field Test: Open field box was made up of the perplex acrylic material with the 16 squares of 18 × 18 cm size marked on the floor and the surrounding wall prevents the escaping of rodent⁸. The center square (18 × 18 cm) was marked in the middle of open field⁹. Albino Rats, both control and treated groups were placed in the peripheral side. Locomotion is observed by counting the entries in the center and peripheral squares for 10 minutes. The test was performed after 30th and 60th days of dosing¹⁰.

Light and Dark Test: Crawly and Goodwin (1980) developed this test for the determination of anxiolytic activity of drugs¹¹. The apparatus comprised of one third of dark box and two third of

light box lightened with 700 LUX. All groups of animals was introduced to the light compartment as by ¹². The percent time spent in the light compartment and transitions made **At 10 min** between two compartments was observed for all groups of animals ¹³.

RESULTS: Various behavioral tests were used to determine the anxiolytic activity of *Argentum nitricum*. One way ANOVA was used for the analysis of data, while post hoc analysis was done by using tuckey test. The comparison in effects within *Argentum nitricum* treated group on day 30th and 60th was analyzed by using paired sample test.

Holepoking Activity: The results were significant on day 30th between groups and within groups ($F = 2.945$, $df = 2, 27$, $p < 0.05$) and highly significant results on day 60th between groups and within groups ($F = 39.795$, $df = 2, 27$, $p < 0.001$). Post hoc analysis by Tukey test showed that *Argentum nitricum* treated mice showed non-significant decrease in head dips on day 30th as well as on day 60th when compared with escitalopram and significant decrease in head dips ($p \leq 0.05$) on day 30th, highly significant decrease in head dips on day 60th ($p \leq 0.001$) in comparison with control. Analysis by paired samples test showed highly significant decrease ($p < 0.001$) in the head dips on day 60th as compared to day 30th within *Argentum nitricum* and escitalopram treated groups.



GRAPH 1: EFFECT OF ARGENTUM NITRICUM ON HOLE POKING ACTIVITY IN MICE

n = 10 Values are mean \pm standard deviation

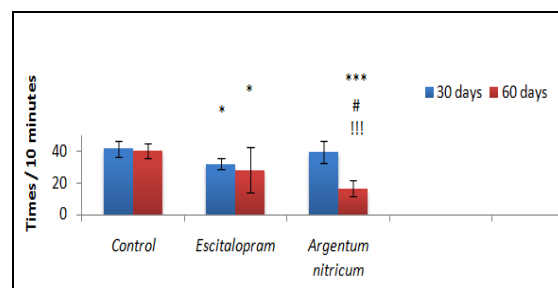
* $p \leq 0.05$ = Significant difference as compared to control

*** $p \leq 0.001$ = Highly significant difference as compared to control

!!! $p \leq 0.001$ = Highly significantly different as compared to one month (30 days) values

Home Cage Activity: *Argentum nitricum* treated group showed highly significant results in cage crossing activity on day 30th between groups and within groups ($F = 4.493$, $df = 2, 27$, $p \leq 0.01$), on day 60 between groups and within groups ($F =$

12.06, $df = 2, 27$, $p \leq 0.001$). Post hoc analysis by Tukey test showed that animals treated with *Argentum nitricum* showed insignificant increase in cage crossings on day 30th and significant decrease ($p \leq 0.05$) on day 60th when compared with standard drug escitalopram and insignificant decrease in cage crossings on day 30th and highly significant decrease ($p \leq 0.001$) on day 60th in comparison with control. Paired sample test analysis showed that cage crossing activity was decreased highly significantly by *Argentum nitricum* on day 60th than as compared to day 30th ($p < 0.001$) and insignificant decrease in escitalopram treated group on day 60th than as compared to day 30th.



GRAPH 2: EFFECT OF ARGENTUM NITRICUM ON HOME CAGE ACTIVITY IN RAT

n = 10 Values are mean \pm standard deviation

* $p \leq 0.05$ = Significant difference as compared to control

*** $p \leq 0.001$ = Highly significant difference as compared to control

$p \leq 0.05$ = Significant difference as compared to standard

!!! $p \leq 0.001$ = Highly significantly different as compared to one month (30 days) values

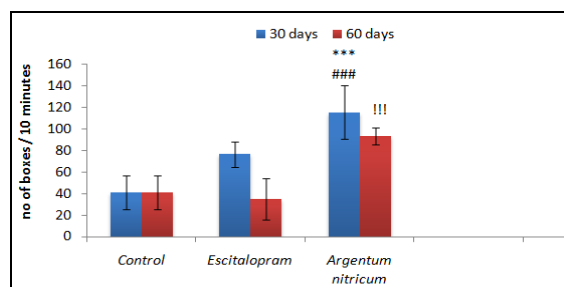
Open Field Activity (Central Square Crossing):

Highly significant results were seen in open field activity in the center squares on day 30th between groups and within groups ($F = 22.407$, $df = 2, 27$, $p \leq 0.001$), and on day 60th between groups and within group ($F = 6.734$, $df = 2, 27$, $p \leq 0.001$) by using one way ANOVA. Post hoc analysis by Tukey test showed that *Argentum nitricum* treated group showed highly significant increase in central square crossings in open field ($p \leq 0.001$) and insignificant increase on day 60th in comparison with standard as well as when compared with control. Paired sample test showed highly significant decrease in central squares crossings on day 60th when compared with day 30th ($p \leq 0.001$) within *Argentum nitricum*.

Open Field Activity (Peripheral Squares Crossings): Moderately significant results were observed in open field activity in the peripheral

squares on day 30th between groups and within groups ($F = 4.412$, $df = 2, 27$, $p \leq 0.01$) and highly significant results on day 60th between groups and within groups ($F = 24.812$, $df = 2, 27$, $p \leq 0.001$) by using one way ANOVA.

Post hoc analysis by Tukey test showed that animals treated with *Argentum nitricum* insignificantly increases peripheral squares crossing in comparison with standard on day 30th but moderately significant increased on day 60th ($p \leq 0.01$) while it showed insignificant increase in peripheral squares crossing on day 30th and significant increase on day 60th ($p \leq 0.05$) in comparison with control. Paired sample test, showed significantly decreased peripheral squares crossings on day 60th when compared with day 30th ($p \leq 0.05$) within *Argentum nitricum* treated group and insignificant decrease in peripheral squares crossings within escitalopram treated group.



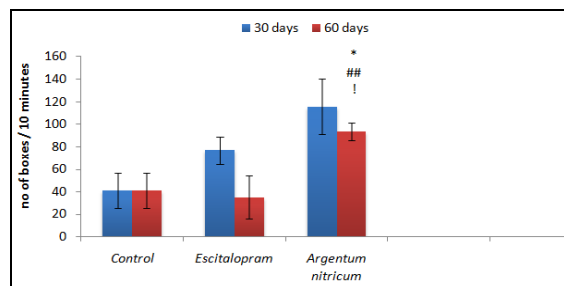
GRAPH 3: EFFECT OF ARGENTUM NITRICUM ON OPEN FIELD (CENTRAL SQUARES CROSSING) ACTIVITY IN RATS

n = 10 Values are mean \pm standard deviation

*** $p \leq 0.001$ = Highly significant difference as compared to control

$p \leq 0.001$ = Highly significant difference as compared to standard

!!! $p \leq 0.001$ = Highly significantly different as compared to one month (30 days) values



GRAPH 4: EFFECT OF ARGENTUM NITRICUM ON OPEN FIELD (PERIPHERAL SQUARES CROSSING) ACTIVITY IN RATS

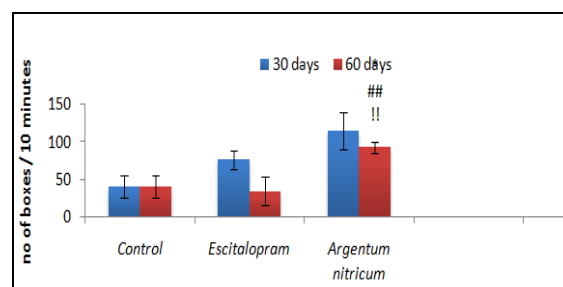
n = 10 Values are mean \pm standard deviation

* $p \leq 0.05$ = Significant difference as compared to control

$p \leq 0.01$ = Moderately significant difference as compared to standard

! $p \leq 0.05$ = Significantly different as compared to one month (30 days) values

Open Field (Total Crossings): Highly significant results in open field activity *i.e.* the total squares crossings on day 30th between groups and within groups ($F = 5.607$, $df = 2, 27$, $p \leq 0.001$), highly significant results on day 60th between groups and within groups ($F = 20.557$, $df = 2, 27$, $p \leq 0.001$) by using one way ANOVA. Post hoc analysis by Tukey test showed that animals treated with *Argentum nitricum* showed insignificant increase in total squares crossing on day 30th but moderately significant increase ($p \leq 0.01$) on day 60th in comparison with standard drug and insignificant increase in total squares crossing on day 30th, significant increase on day 60th ($p \leq 0.05$) in comparison with control. Paired sample test, showed moderately significant decrease in total squares crossings on day 60th when compared with day 30th ($p \leq 0.01$) within *Argentum nitricum* treated group, and insignificant decrease in total squares crossings on day 60th when compared with day 30th within escitalopram treated group.



GRAPH 5: EFFECT OF ARGENTUM NITRICUM ON OPEN FIELD (TOTAL SQUARES CROSSING) ACTIVITY IN RATS

n = 10 Values are of mean \pm standard deviation

* $p \leq 0.05$ = Significant difference as compared to control

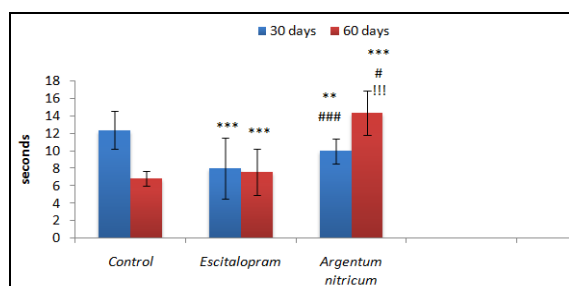
$p \leq 0.01$ = Moderately significant difference as compared to standard

!! $p \leq 0.01$ = Moderately significantly different as compared to one month (30 days) values

Light and Dark Test (% Time Spent in Light Compartment):

Highly significant results in percent time spent in light compartment on day 30th between groups and within groups ($F = 75.47$, $df = 2, 27$, $p \leq 0.001$), moderately significant results on day 60th between groups and within groups ($F = 75.866$, $df = 2, 27$, $p \leq 0.001$). Post hoc analysis by Tukey test showed that animals treated with *Argentum nitricum* showed highly significant increase on day 60th ($p \leq 0.001$) in comparison with control and highly significant increase in percentage time spent in light compartment on day 30th ($p \leq 0.001$), significant increase on day 60th ($p \leq 0.001$), significant increase on day 60th ($p \leq 0.001$), significant increase on day 60th ($p \leq 0.001$).

≤ 0.05) in comparison with standard and moderately significant decrease in percentage time spent in light compartment ($p \leq 0.01$) on day 30th. Paired sample test, showed that *Argentum nitricum* treated group showed highly significant increase in % time spent in light compartment on day 60th when compared with day 30th ($p \leq 0.001$) while escitalopram showed insignificant increase on day 60th in comparison with day 30.



GRAPH 6: EFFECT OF ARGENTUM NITRICUM ON % TIME SPENT IN LIGHT COMPARTMENT

n = 10 Values are mean \pm standard deviation

** $p \leq 0.01$ = Moderately significant difference as compared to control

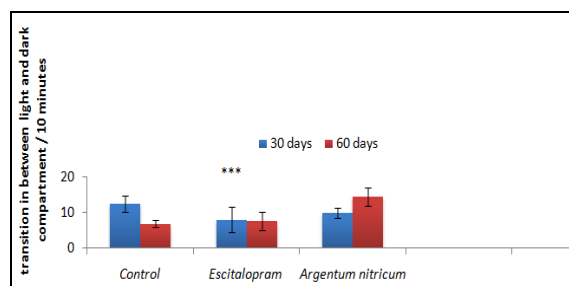
*** $p \leq 0.001$ = Highly significant difference as compared to control

$p \leq 0.05$ = Significant difference as compared to standard

$p \leq 0.001$ = Highly significant difference as compared to standard

!!! $p \leq 0.001$ = Highly significantly different as compared to one month (30 days) values

Light and Dark Test (Transitions between Compartments): Highly significant results in transitions in between two compartments on day 30th between groups and within groups ($F = 13.494$, $df = 2, 27$, $p \leq 0.001$), insignificant results on day 60th between groups and within groups ($F = 0.705$, $df = 2, 27$, $p = IS$).



GRAPH 7: EFFECT OF ARGENTUM NITRICUM ON TRANSITIONS BETWEEN LIGHT AND DARK COMPARTMENT (NUMBER OF TRANSITIONS / 10 MIN)

N = 10 Values are mean \pm standard deviation

*** $p \leq 0.001$ = Highly significant difference as compared to control.

Post hoc analysis by Tukey test showed that animals treated with *Argentum nitricum* showed insignificant increase in transitions between two compartments in comparison with standard on day

30th and day 60th and insignificant decrease on day 30th and insignificant increase on day 60th in comparison with control. Paired sample test, showed that *Argentum nitricum* insignificantly increased and Escitalopram insignificantly decreased transitions between light and dark compartment on day 60th when compared with day 30th.

DISCUSSION: In present study, we observed that *Argentum nitricum* after 30 and 60 days of dosing decreased number of head dips and home cage activity that reflects its anxiolytic potential. *Argentum nitricum* is used for extremely anxious patient as reported by ¹⁴. The possible mechanism is through CNS depression. The crucial thing is the dose, because an increase in dose could lead to accumulation of silver particles at the level of glial cells and spinal cord ^{15, 16}. Zhang et al., (2013) ¹⁷ had reported that after induction of silver nano particles, rats have shown anxiolytic effect supporting our finding.

Another finding was that the central and peripheral square crossings were found to after administration of *Argentum nitricum* supporting its anxiolytic activity. The effect was more pronounced after 30 days of dosing. Increase in central square crossing confirms its anxiolytic potential and possibly this action is through the release of neurotransmitter especially nor-epinephrine and dopamine. The total number of crossing were decreased after 60 days of dosing, although still better as compared to control, showing that prolonged dosing may affect the concentration of neurotransmitter and/or down regulate the receptors.

This hypothesis is supported by ¹⁷ who reported that after prolonged administration of silver nano particle both rearing and locomotion was decreased. Our study has also shown that total crossings were decreased after 60 days of dosing as compared to day 30th. This further supports our suggestion that *Argentum nitricum* should be given in low doses and duration of treatment needs to be clearly defined.

The time spent in light compartment and the number of transitions between light and dark compartment were found increased especially after 60 days of dosing. This proves its anxiolytic

activity which was found pronounced after chronic dosing. The increase in transitions may be due to increase in concentration of norepinephrine and dopamine as reported earlier^{18, 19}.

CONCLUSION: At the end of this study novel results had been produced and we had reached to the conclusion that *Argentum nitricum* possess a potent anxiolytic activity and can be used for treating anxiety. It possesses better results in comparison with the standard drug escitalopram, but it is suggested that low doses of *Argentum nitricum* should be administered because of its potential to produce adverse effect.

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CONFLICT OF INTEREST: There is no potential conflict of interest in the research.

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