



Antidepressant Potential of *Vernonia Anthelmintica* Willd Extracts in Experimental Animals

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Abstract

The present study was carried out to evaluate the antidepressant potential of *Vernonia anthelmintica* willd seed extracts in experimental animals. Methanolic and aqueous extract of *Vernonia anthelmintica* seeds were carried out by soxhlet extraction and maceration technique respectively. The extracts were administered at dose levels of 125, 250 and 500 mg/kg orally. The antidepressant activity was evaluated by Tail suspension test and Forced swim test. Locomotor activity was carried out by activity cage meter. Fluoxetine at 10 mg/kg was used as standard antidepressant drug. *Vernonia anthelmintica* extracts showed the stimulant effect in locomotor activity. The duration of immobility time is reduced in dose dependent manner after repeated treatment with *Vernonia anthelmintica* extracts for 7 days in Forced swim test and Tail suspension Test. In the present study, *Vernonia anthelmintica* methanolic and aqueous extract showed significant antidepressant activity in dose dependent manner. The phytoconstituents present in the *Vernonia anthelmintica* may be responsible for its antidepressant activity.

Keywords: Anti-depressant, fluoxetine, forced swim test, tail suspension test, *Vernonia anthelmintica*

1. Introduction

Depression is a life-threatening mental disorder with high morbidity and mortality [1]. Depression is a common, debilitating chronic recurrent syndrome, often refractive to drug treatment affecting quality of life and overall productivity. It has been reported that one-year prevalence rate is about 5% and recurrence rate is up to 85% [2, 3].

The World Health Organization estimates that depression is now the fourth most important cause worldwide of loss in human disability. Although pharmacotherapy of depression includes battery of drugs, their efficacy is unsatisfactory, they exert multiple unwanted side effects and also their antidepressant mechanism is not completely understood [4, 5].

However, it is clear that dysfunction of the brain monoamine systems [e.g., serotonin (Sero), dopamine (DA) and norepinephrine (NE)] is likely to have a role in the pathophysiology of depressive disorders [6]. There is change in these neurotransmitter levels in patients having major depression [4].

Current therapy for antidepressants include different class of compound like serotonin reuptake inhibitors, tricyclic antidepressants etc. all exert their antidepressant effect by increasing the levels of monoamines Sero and/or NE. They have slow onset and have severe side effects [7, 8]. Therefore, there is urgent need to explore more promising antidepressant drugs [9]. Recently, the usage of traditional herbs has provided us a prospective alternative in the treatment of depression because of its better compliance and lower side effects [10–12]. Plant

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sources such as *Withania somnifera*, *Bacopa monniera* and St. John's wort extract have been reported to have antidepressant activity and can be effective therapeutic alternatives for treatment of depression. Although, several classes of antidepressants are currently being used, due to clinical limitations and adverse effects there is critical interest in development of efficient and safe drugs for treatment of depression [4, 13].

The *Vernonia anthelmintica* Linn. (VA) belonging to family Asteraceae is known as Purple fleabane in English, Kalijiri in Marathi. The plant is widely distributed throughout India and is common in waste places near villages. The dried seeds of this plant are extensively available in market of India. Dried seeds, leaves and roots of VA are reported to be used traditionally in various ailments, seeds of this plant are acrid and astringent to the bowels and are used as tonic, stomachic, diuretic, anthelmintic, purgative, ulcers, asthma, intestinal colic, dysuria, kidney troubles, "vata" and "kapha" (Ayurveda) and inflammatory swellings. As the scientific name of the plant indicates, it is a valuable medicine as anthelmintic. It is useful in thread worm infections. The seeds are black of a bitter taste [14, 15].

The seed contains about 20–30% oil of which more than 70–74% is epoxy acid or vernolic acid. The seeds found to contain sterols, flavon-glucosides, vernodaline, methylvernosterol, butein, chalcone, β -amyrin, β -sitosterol, β -D-glucoside, Vernolic acid, stigmasterol, flavonoids. *V. anthelmintica* fruits are boiled with black pepper and garlic and its decoction is given in diarrhoea. Infusion of the plant is given in stomach worms (round and tape worms) [16]. *V. anthelmintica* seeds are used as anthelmintic for the treatment of gastrointestinal trichostrongylids in small ruminants [17]. Antifilarial activity of *C. anthelminticum* seed extracts on *Setaria cervi* has been reported by Singhal et al. [18]. Reports on the chemical constituents of various plants and their pharmacology suggest that plants containing flavanoids and tannins possess activity against many Central nervous system disorders. Flavanoids present in the *Hypericum perforatum* and tannins present in the *Embllica officinalis* are responsible for antidepressant action. So these components present in the *Vernonia anthelmintica* extract may provide scientific background to be useful in central nervous system disorders. Hence the present study was designed to investigate the effects

of *Vernonia anthelmintica* extracts on various models of depression [19].

2. Materials and Methods

2.1 Plant Material and Preparation of Extract

The plant material seeds of *Vernonia anthelmintica* (VA) were collected from the local market of Mumbai. The seeds were authenticated from Dr Ganesh Iyer, Department of Botany, Ruia College, Matunga, Mumbai. Seeds were washed properly then air dried and finally dried in the oven at 40–50°C. After ensuring the seeds were completely dried, they were powdered to a fine size. The powdered plant material is defatted with pet ether and then successively extracted with methanol in soxhlet extractor, the methanolic extract of *V. anthelmintica* (VA ME) obtained. The aqueous extract of *Vernonia anthelmintica* (VA AQ) was prepared by maceration technique. Both methanolic and aqueous extract of *Vernonia anthelmintica* were filtered through vacuum filter, the filtrates were further concentrated in vacuum evaporator which were used for the further studies.

2.2 Chemicals

The *Vernonia anthelmintica* Methanolic extract (VA-ME) at dose levels of 125, 250 and 500 mg/kg and the *Vernonia anthelmintica* Aqueous extract (VA-AQ) at dose levels of 125, 250 and 500 mg/kg were given orally to animals for the study. Fluoxetine hydrochloride was obtained from Sigma Aldrich and used as standard antidepressant drug at 10 mg/kg dose for the present study.

2.3 Animals

Male Swiss albino mice weighing between 20–25 g were used for the study. The animals were procured from Haffkine Biopharmaceuticals, Parel Mumbai. The animals were housed in polypropylene cages and were maintained at $25 \pm 2^\circ\text{C}$ with relative humidity $55 \pm 10\%$ and 12:12 hours light: dark cycle. Animals were acclimatized to experimental room environment before one week, also provided standard pellet diet and *water ad libitum*. Animals were acclimatized for at least one week before using them for experiments and exposed only once to every experiment. The experimental protocols

were approved by the Institutional Animal Ethical Committee (IAEC) of Institute of Chemical Technology, Mumbai.

3. Experimental Protocol

3.1 Locomotor Activity

Locomotor activity in mice was measured using actophotometer. The digital data being displayed on the front panel meters as an ambulatory movements of animal. Mice were allowed to acclimatize to the observation chamber for a period of 2 min. Locomotion was expressed in terms of total photobeam counts per 10 min per animal. Activity cages and floor surfaces were thoroughly cleaned with 70% ethanol between tests. All the drugs were given via the oral route once a day for 7 days. Locomotor activity was conducted 60 min after the first oral dose and 24 h after repeated treatment for 7 days with drugs [20, 21].

3.2 Forced Swim Test - (FST)

Behavioral despair was proposed as a model to test antidepressant activity by Porsolt et al [22].

Mice were forced to swim in a restricted space i.e. in a glass vessel containing water of 15-20 cm with temperature of water maintained at around 25°C. On the day of experiment, animals were treated with test extract 60 min prior to test and then the mice were forced to swim in a glass vessel for 6 min. Mice showed initial vigorous-escape related activity for 2 min. Following these 2 minutes animal started to show immobility. The total duration of immobility for 4 min was noted. The immobility observed as the animal shows minimum movement for keeping its head above water or floating without struggling. In some cases animal shows climbing activity which was also be noted. After completion of 6 min test period, mice were towel dried and returned to their cages. The test was carried out after acute treatment and to investigate the subchronic effect the test is repeated after 7 days of treatment with test extracts [23, 24].

3.3 Tail Suspension Test - (TST)

Tail suspension test is a validated animal model to screen anti-depressant drugs. Animals were treated with test extracts 60 min before the test. Then mice were

suspended by their tail with the help of adhesive tape which was placed about 1 cm from the tip of the tail. Mice were suspended by tail at height of around 58 cm from the surface. After suspending mice showed initial escape like-struggling activity nearly about 2 min. Then the animal shows immobility which was measured for 4 min. The mice said to be immobile when it did not show any escape like activity and hanged passively. The test was carried out after acute treatment and to investigate the subchronic effect the test is repeated after 7 days of treatment with test extracts [20, 24].

4. Statistical Analysis

Data were expressed as Mean \pm S.E.M. Statistical analysis of data was done by One-way ANOVA, followed by Dunnett's test for comparison between control and test groups by using Graph Pad Prism 4 (San Diego, CA) software. The p value of < 0.05 was considered to be statistically significant.

5. Results

5.1 Effect of VA Extracts on Antidepressant and Locomotor Activity after Acute Treatment

Antidepressant activity was carried out in mice treated with VA ME and VA AQ extracts orally 60 min before the forced swim test and Tail suspension test and the test was repeated after the 7 days dosing of test extracts.

5.1.1 The Effect of Acute Treatment of VA ME and VA AQ on Immobility Time in the Locomotor Activity by Actophotometer

Each value represents Mean \pm SEM ($n = 6$), significantly different from vehicle control (ns- non significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Locomotor activity was depicted in the Fig. 1A and 1B after acute treatment with *Vernonia anthelmintica* methanolic extract and aqueous extract respectively. Analysis was carried out by one way ANOVA. The results showed that there was no significant ($p < 0.05$) difference in total locomotor activity at dose of 125 mg/kg of VA ME and VA AQ extracts. VA ME and VA AQ at 500 mg/kg ($p < 0.05$) showed significant difference in locomotor activity.

5.1.2 The Effect of Acute Treatment of VA ME and VA AQ on Immobility Time in the FST

The immobility time in forced swim test was measured after acute treatment with VA ME and VA AQ extracts and shown in Fig. 2A and 2B respectively. One way ANOVA showed significant difference in immobility time in FST on mice among experimental groups. VA ME and VA AQ at 250 mg/kg ($p < 0.05$), 500 mg/kg ($p < 0.05$) treated had significantly decreases immobility time compared with vehicle treated mice in the FST. This effect was similar to that of Fluoxetine 10 mg/kg ($p < 0.05$), an SSRI type antidepressant drug

served as positive control and VA ME and VA AQ at 125 mg/kg ($p < 0.05$) group showed less significant effect.

Each value represents Mean \pm SEM ($n = 6$), Significantly different from vehicle control (ns- non significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

5.1.3 The Effect of Acute Treatment of VA ME and VA AQ on Immobility Time in the TST

Each value represents Mean \pm SEM ($n = 6$), significantly different from vehicle control (ns- non significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

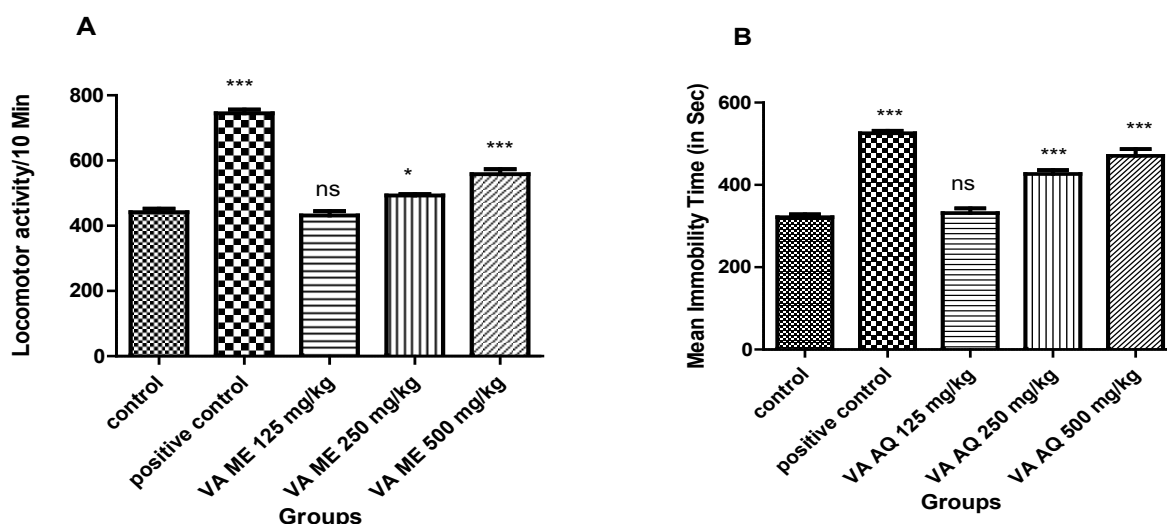


Fig. 1A and 1B. Effect of *V. anthelmintica* methanolic and aqueous extract (after single dose) on locomotor activity per 10 min in activity cage model.

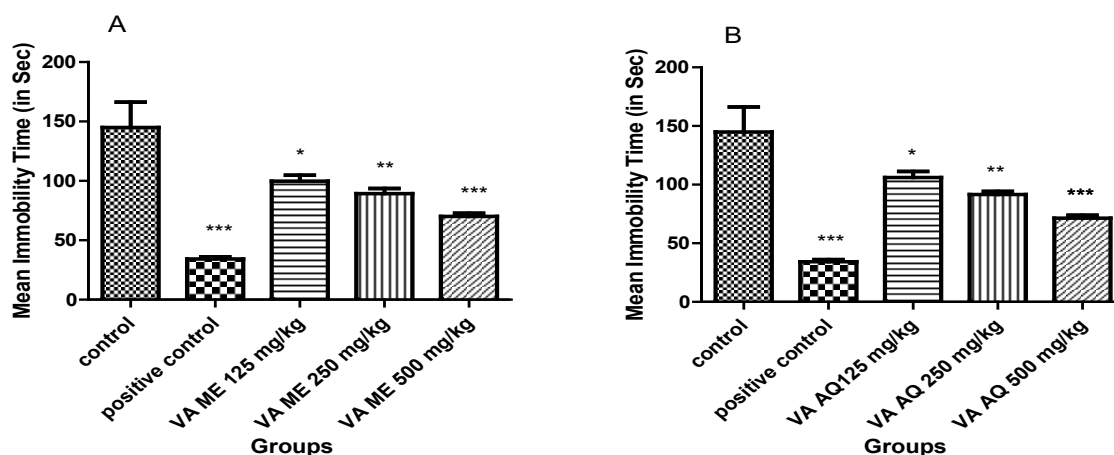


Fig. 2A and 2B. Effect of *V. anthelmintica* methanolic and aqueous extract (after single dose) administration on mean immobility time in forced swim test.

Results for VA ME and VA AQ were shown in Fig. 3A and 3B respectively. After acute administration, VA ME at 250 mg/kg ($p < 0.05$), 500 mg/kg ($p < 0.05$) treated mice showed significant decrease in mean immobility time as compared to the control group. The positive control group treated with fluoxetine showed comparable results. The mice treated with VA AQ at 125 mg/kg dose showed non significant effect in lowering the mean immobility time.

5.2 Effect of Repeated Treatment of VA ME and VA AQ on Anti-depressant Activity and Locomotor Activity

The tests were carried out after acute treatment and to investigate the subchronic effect, mice were treated

with test extracts for 7 days followed by testing for antidepressant and Locomotor activity

5.2.1 Effect of Repeated Treatment with VA ME and VA AQ on Immobility Time in FST

Each value represents Mean \pm SEM ($n = 6$), significantly different from vehicle control (ns- non significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

The results for VA ME and VA AQ was shown in Fig. 4A and 4B respectively. VA ME at 125 mg/kg ($p < 0.05$), 250 mg/kg ($p < 0.05$), 500 mg/kg ($p < 0.05$) treated mice had significantly decreases immobility time compared with vehicle treated mice in the FST. This effect was similar to that of Fluoxetine 10 mg/kg

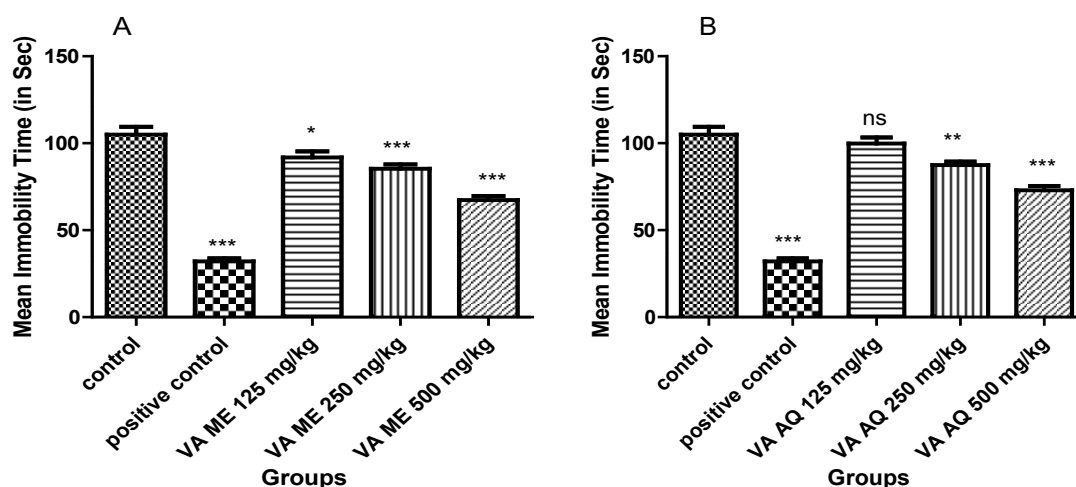


Fig. 3A and 3B. Effect of V. anthelmintica methanolic and aqueous extract (after single dose) administration on mean immobility time in Tail Suspension test.

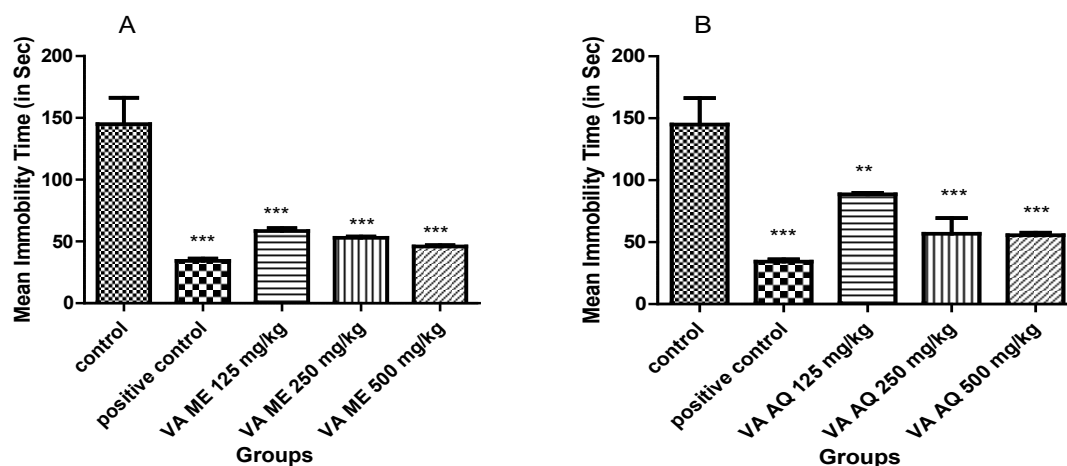


Fig. 4A and 4B. Effect of V. anthelmintica methanolic and aqueous extract after repeated dose (for 7 days) administration on mean immobility time in Forced swim test.

($p < 0.05$), an Selective Serotonine Reuptake Inhibitors (SSRI) type antidepressant drug served as positive control and VA AQ group also decreases immobility time in FST.

5.2.2 Effect of Repeated Treatment with VA ME and VA AQ on Immobility Time in Tail Suspension Test

Each value represents Mean \pm SEM ($n = 6$), Significantly different from vehicle control (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

The antidepressant effect of VA extracts in Tail suspension test after repeated treatment with VA ME and VA AQ for 7 days was given in Fig. 5A and 5B respectively. The VA ME and VA AQ at dose level of 125, 250 and 500 mg/kg groups showed significant activity by

decreasing the immobility time as compared to that of control group in TST. This effect was comparable to that of positive control Fluoxetine.

5.2.3 Result of Locomotor Activity after Repeated Treatment with VA ME and VA AQ Extracts

Each value represents Mean \pm SEM ($n = 6$), significantly different from vehicle control (ns- non significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

The result of locomotor activity of VA ME and VA AQ was depicted in the Fig. 6A and 6B respectively. After repeated treatment with *V. anthelmintica* extracts VA ME and VA AQ at 250 mg/kg ($p < 0.05$) and 500 mg/kg ($p < 0.05$) showed significant difference in locomotor activity. VA AQ at 125 mg/kg ($p < 0.05$) was found to be non significant.

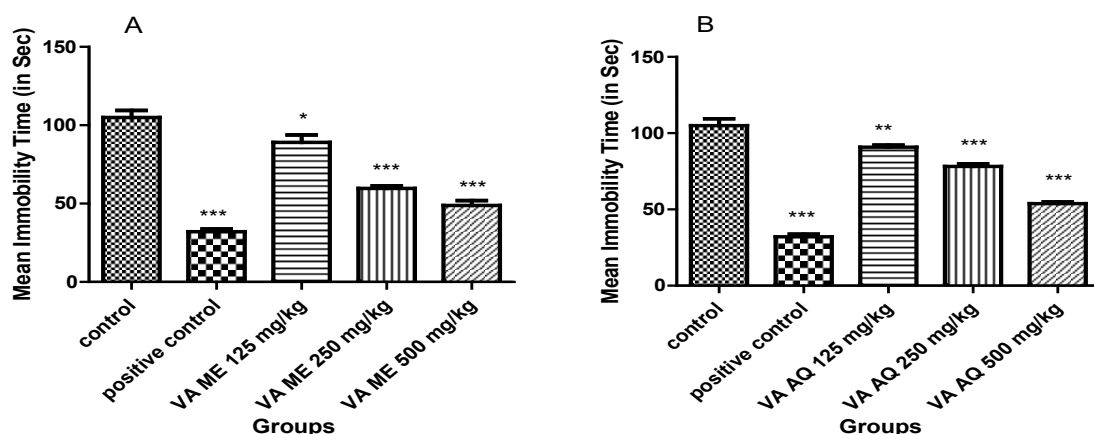


Fig. 5A and 5B. Antidepressant activity of *V. anthelmintica* methanolic and aqueous extract (Repeated dosing for 7 days) in Tail suspension Test.

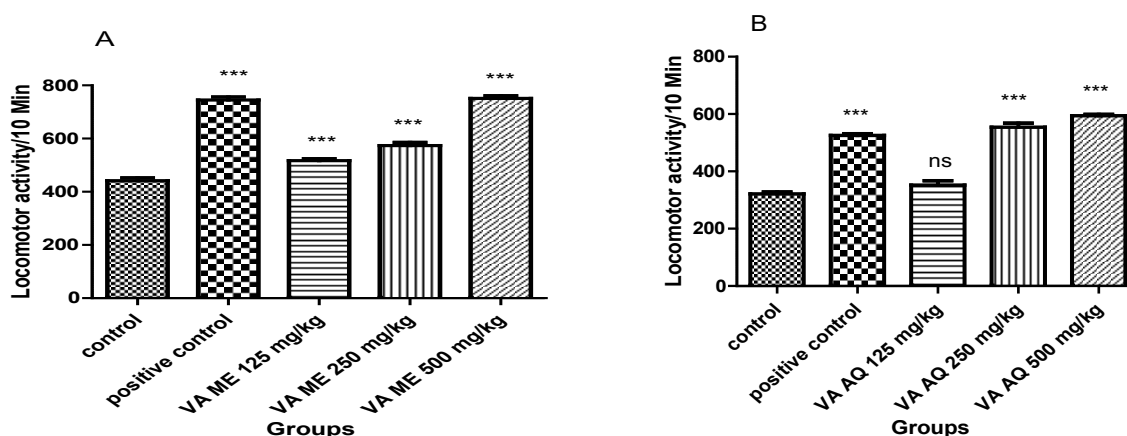


Fig. 6A and 6B. Effect of *V. anthelmintica* extracts (Repeated dosing for 7 days) in activity cage model.

6. Discussion

In the present study FST and TST were carried out which are widely used, accepted and simple models for screening the antidepressant activity. The immobility is noted as a measure of antidepressant action also swimming behaviour corresponds to the behavioural despair as seen in human depression [25]. It was reported that the major depressive disorder involves disturbances of life style, emotional changes, autonomic and endocrine functions affecting about 20% of population [24].

In the present study *in vivo* animal models FST and TST were used to study the antidepressant potential of VA ME and VA AQ extracts. The oral administration of VA ME and VA AQ extracts were found to be effective in reducing the mean immobility time in mice, indication potential as antidepressant activity.

It is accepted that immobility seen in rodents during swimming reflects behavioral despair as seen in human depression and that the antidepressant drugs are able to reduce the immobility time in mice [25].

The locomotor activity of animal was performed by using Activity cage meter. It was found that single dose administration of VA ME and VA AQ showed stimulant effect at dose levels of 250, 500 mg/kg while 125 mg/kg was found to be non significant ($P < 0.05$). The result obtained from repeated administration of VA ME and VA AQ on mice in locomotor activity showed that VA ME has significant locomotor activity as compared to VA AQ. VA AQ showed significant locomotor activity at higher dose levels of 250, 500 mg/kg while 125 mg/kg was found to be non significant.

The Forced swim test is a simple, reliable and specific model to screen antidepressant activity. This model shows positive response to acute antidepressant treatments. The mean duration of immobility time is noted as a measure of antidepressant activity. Many antidepressants and compounds possessing antidepressant activity shows decrease in immobility time [26, 27]. The oral administration of VA ME and VA AQ showed positive effect in reducing the mean immobility time in FST, which is evident from the significant reduction in the immobility time by treatment of VA extracts [28].

We have explored the acute and subchronic antidepressant effects of VA using the FST and TST animal models. After the acute treatment with VA

extracts, the decrease in mean immobility time was observed in the FST and TST tests. The mice were treated with extracts for 7 days to investigate the subchronic effect of VA extracts and then subjected to FST and TST. In the FST, the dose level of 250 and 500 mg/kg showed significant reduction in immobility time as compared to the control group, while 125 mg/kg treated group was found to be less significant. In the TST, the immobility time was greater at 500 mg/kg. From the results it was observed that VA ME was found to be more active in reducing the immobility time as compared to VA AQ.

Fluoxetine exhibited the characteristic behavioral effects of a selective serotonin reuptake inhibitor in the modified forced swim test, i.e. a decrease in immobility coupled with an increase in swimming behavior. Although the precise mechanism involved in the observed antidepressant activity is not yet clear. The experimental observations suggest a possible direct or indirect facilitation of the central serotonergic transmission for the species studied.

Preliminary phytochemical analysis carried out with the methanol extract of this species revealed the presence of flavonoids, tannins, saponins and anthraquinones. Since antidepressant effects have been observed in several flavonoids from *Hypericum perforatum*, it is possible that these polyphenolic substances might be responsible, at least in part, for the antidepressant activity in study. Thus the flavonoids may be responsible for the said antidepressant activity of VA [25, 29].

Further studies should be carried out to correlate the pharmacological activities with the chemical constituents. Several compounds of distinct nature which are present in the plant are relevant for the behavioural effects of this plant.

The study of *Vernonia anthelmintica* in experimental animals showed the decrease in immobility time in dose dependent manner suggesting its antidepressant potential.

7. Conclusion

From the above study, we can conclude that methanolic extract of *Vernonia anthelmintica* show a significant antidepressant activity in TST and FST models of depression. Further research is required to gain closer insights into the exact mechanism of its action.

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