INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

EVALUATION POTENTIAL OF VALERIAN ROOTS EXTRACT AND VALEPOTRIATES ON BEHAVIORAL TESTS IN MICE

Edvaldo Rodrigues de Almeida[1*], Haroudo Sátiro Xavier[2], Aluizio Roberto da Silva[1], Thais Malheiros Chaves[3], Adelmo Cavalcanti Aragão-Neto[3]
[1]Department of Antibiotics of the Federal University of Pernambuco, Brazil[1]
[2]Department of Pharmacognosy of the Federal University of Pernambuco, Brazil[2]
[3]Department of Odontology of the Federal University of Pernambuco Brazil[3]

Article Received on: 16/03/2011 Revised on: 28/04/2011 Approved for publication:17/05/2011

* Edvaldo Rodrigues de Almeida, E-mail: edvaldo.ra@gmail.com

ABSTRACT
This study was to evaluate the effect of Valerian extract marketed in Recife PE Brazil and Valepotriates and their actions in the CNS in mice. Intraperitoneal administration were used to assess the hypnotic effect (Sodium Pentobarbital-Induced Sleeping Time), anxiolytic (Elevated plus-maze, Hole-board, Marble burying), and the observation of possible anticonvulsant activity (seizures induced by Pentylenetetrazol), using doses of 50, 100 and 200 mg / kg for the extract and 12 mg/kg for Valepotriates**.** Data indicate an anxiolytic action, and hypnotic only by the intraperitoneal route, but oral administration showed no significant effect on the behavioral tests (data not shown). This study demonstrated an anxiolytic and hypnotic action extract of Valeriana officinalis, as well as the Valepotriates at a dose of 12 mg/kg.
KEYWORDS: Anti-Anxiety, Behavior, Valerian officinalis, Mice

INTRODUCTION

The Valeriana officinalis (Val) is an herbaceous perennial plant belonging to the Valerianaceae family. Is widely distributed throughout Europe and Asia and the sampling period is between autumn and spring. Immediately after harvest, the rhizomes and roots should be dried at a temperature of 40°C to prevent degradation of the active components[1]. Valerian is a relatively well-studied herb, and several groups of identified constituents seem to explain some of effects of the whole herb. These include the valepotriates (Valep), a group of iridoid glycosides comprising upwards of 0.5-2% in *V. officinalis,* including the Valtrate, Isovaltrates, Didrovaltrates, and Valerosidate. The valtrates and isovaltrates are stated to comprise upwards of 90% of the valepotriates. V. wallachii and V. edulis contain upwards of 6% and 8% valepotriates, respectively. Important volatile constituents in V. officinalis include the monterpene borneol, the sesquiterpene valerenal, and carboxylic compounds such as esters of valerianic and isovaleric acid. The *European Pharmacopoeia* states that V. officinalis should contain no less than 5 mL/kg of essential oil. Non-volatile cyclopentane sesquiterpenes include valerenic acid and its derivatives. Valerian also contains Pyridine Alkaloids such as Actinidine, Chatinine, Skyanthine, Valerianine and Valerine, as well as Lignans, Amino acids, Caffeic and Chlorogenic acids, Beta-Sitosterol, Methyl 2-Pyrrolketone, Choline, Tannins, Gum and a Resin. The characteristic odor of Valerian is stated to be due to Valerianic and Isovalerianic acid in the volatile oil[2, 3]. The activity of valerian is as a sedative, it is also anxiolytic, spasmolytic, relaxing, soothing and anti-ulcerogenic, the main effect of which is reducing time to induce sleep[4]. Valerenic acids are present (Valerenic, Acetoxymanoyl-Valerenic and Hidroxivalerenic) have shown a decrease in the degradation of γ-aminobutyric acid (GABA). The increased concentration of GABA in the synaptic cleft is one of the factors responsible for the sedative properties of the plant [5-10]. It seems that the Valerenic acid acts similarly to Benzodiazepines, by binding to specific subunits of the GABAA receptor complex. The stimulation of this receptor directly opens chloride channels producing a neural inhibition[8, 10]. Another mechanism that may contribute to the sedative properties of valerian is high levels of glutamine in the extract, which crosses the blood-brain barrier and can be converted into GabaA nerve terminals [11, 12]. The aim of this study was to investigate the efficacy and safety of Valerian extract for anxiety through the herb sold in the city of Recife PE Brazil. This study aims to evaluate the effect of anxiolytic, anticonvulsant, and even the sleep-

enhancing properties, using various behavioral tests, such as elevated Plus-maze, Marble-Burying Test, Board-Hole test, Sodium Pentobarbital-Induced Sleeping Time (PBS) and Pentylenotetrazole-Induced Convulsion (PTZ). Researchers, to test the anxiolytic effect of drugs on animals[13-17] have frequently used the elevated Plus Maze.

MATERIALS AND METHODS

Animals

Male two-month-old Swiss albino mice, weighing 20–30 g, were used in this experiment. The animals were housed in groups of fifteen[15] per cage, with a light/dark period of 12 hours. They were fed and watered ad libitum. All experiments were conducted between 10:00 am and 4:00 pm. All animals were carefully monitored and maintained in accordance with the ethical recommendation of the Brazilian College of Animal Experimentation (COBEA) and the National Institute of Health Guide for Care and use of Laboratory Animals and approved by the Ethical Committee of the Federal University of Pernambuco (UFPE) protocol number 23076.022.038/2008-11.

Drugs

Diazepam (DPZ, 2 mg/kg, i.p.) was used as the standard anxiolytic drug; Pentobarbital sodium (PBS, 55 mg/kg, i.p.) was used as the standard hypnotic Diazepam (2 mg/kg, i.p.) drug and Pentylenotetrazole (PTZ, 55 mg/kg, i.p.) as the standard convulsant drug an. All drugs were obtained from Sigma Aldrich, Mo, USA, and administered by intraperitoneal route at volume of 0.1 mL/10g BW. Val was administered at doses of 50, 100, and 200 mg/kg BW, and Valep at dose of 12 mg/kg in all experiments.

Extract Preparation and analysis Phytochemical

To obtain the methanol extracts 500 mg of dry material, previously ground, were used. The extraction was done by using increasing polarity solvents (hexane, ethyl acetate and methanol). We then carried out lyophilization, resulting in a yield of 25g of material, which was carefully sealed and stored at-20 °C. Regarding phytochemical analysis, the main compounds present in the roots of Valeriana officinalis L. the valepotriates (Valep) (e.g. Valtrato, Acevaltrato, Didrovaltrato) are Triester, of a Monoterpene alcohol, with the structure of an Iridoid Cyclopenta (c) Pyran with an attached epoxy ring **(**Figure 1**)**. These iridoid esters are unstable and easily form degradation products (eg polymers, Baldrine). The essential oil contains esters of Isovalerianic acid, α-hydroxyisovalerianic, Eugenol, Bornyl acetate, Terpene hydrocarbons and Sesquiterpenes (eg. Valeranone). The Sesquiterpene carboxylic acids (e.g. acid and Acetoxy Valerenic) are stable, non-volatile. These compounds contribute to the sedative effect of the root of V. officilalis[14].

Sodium Pentobarbital-Induced Sleeping Time Test

The mice were divided into six subgroups (15 animals each). Three subgroups received different doses of extract (50, 100, and 200 mg/kg, i.p.), 12 mg/kg Valep and DPZ. After 1 hour, all four groups received 55 mg/kg (IP) of Sodium Pentobarbital (PBS). The time that elapsed between the loss and recovery of the righting reflex was recorded, for control and drug pretreated animals[15].

Pentylenotetrazole-Induced Convulsion Test

The mice were divided into six subgroups (15 animals each). The first subgroup perceived the Pentylenotetrazole (PTZ) (55 mg/kg, i.p.) after saline solution and served as positive control. The test subgroups received the Val at doses of 50, 100, and 200 mg/kg (IP) and 12 mg/kg Valep. After 1 hour, PTZ (55 mg/kg, i.p.) was administered to the animals in each subgroup. The number of mice that exhibited convulsions, the lethal time and the latency to first convulsion was recorded[15].

Marble-Burying Test

Twenty-five clear glass marbles (20 mm diameter) were used for each individual test. Opaque cages (30×36×13 cm) were constructed of smooth, opaque plastic with a vinyl ceiling containing air holes, and a 5 cm layer of sawdust. Mice were placed individually in these cages for 15 minutes (habituation trial) and then returned to their home cage. Twenty-five glass marbles were evenly spaced 5 cm apart on a 5 cm layer of sawdust in the habituation cages. Mice were then reintroduced (each test mouse was returned to the same cage in which they had been habituated**).** Fifteen animals were used in each group. The test groups received the Val at doses of 50, 100, and 200 mg/kg (i.p.) and Valep 12 mg/kg. After 15 minutes, the test was terminated by removing the mice and counting the number of marbles that were more than two-thirds covered with sawdust. After each trial, the sawdust was replaced, and the test apparatus and glass marbles were washed by water and cleaned with 70% alcohol[16].

Hole-Board Test

Exploratory behavior was assessed using the hole-board test. The apparatus consisted of a square plastic plate, 40 cm x 40 cm, 1 cm thick, with 16 holes (diameter 2 cm), regularly spaced on the surface, at 3.5 cm from the edges. The apparatus was elevated to the height of 50 cm, in a dimly illuminated room. The test groups received the Val at doses of 50, 100, and 200 mg/kg and Valep 12 mg/kg (i.p.). Mice were placed in the centre of the plate, and the number of head dips was immediately

counted during two or three consecutive periods of 5 minutes each[17].

Elevated Plus-Maze Test

The elevated plus-maze (EPM) test consisted of two open arms (30 x 5 x 0.25 cm) and two closed arms (30 x 5 x 15 cm) emanating from a common central platform (5 x 5 cm). Two pairs of identical arms were opposite to each other. The entire apparatus was elevated to a height of 40 cm above floor level. At the beginning of the session, a mouse was placed at the centre of the maze, its head facing an open arm and allowed to explore the maze for 5 minutes, and the following parameters were scored: the time spent and number of entries in each type of arms. The plus maze was carefully cleaned with a wet towel after each animal test. The mice were divided into six groups (fifteen animals per group). DPZ (2.5 mg/kg IP) was used as the positive control and Val at doses of 50, 100, and 200 mg/kg and Valep 12 mg/kg (i.p.) in the three remaining groups. All experiments were carried out between 10:00 am and 4:00 pm. After each trial, the EPM apparatus was wiped clean with alcohol (70%) solution [13].

Statistics Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with *post hoc* Turkey's test. $P<0.05$ (n=15) was considered significant. All data are expressed as an arithmetical mean ± S.E.M. All statistical analysis was carried out in the Program Graph Pad Prism.

RESULTS

Figure 1. Phytochemical analysis showing the two main compounds (Valtrate and Valepotriates).

Figure 2 Sodium Pentobarbital-induced sleeps. The effect of pentobarbital sodium-induced sleep is shown in Fig 4. The values, up to 50, 100 and 200 mg/kg of Val, as well as Valepar and DPZ also showed a significant effect were significantly different from the Control group * $p<0.01$. Values are mean ± SEM (n=15).

Figure 3. Pentylenotetrazole-induced convulsion Test. Val and Valep non-inhibited generalized seizures induced by PTZ (55 mg/kg, p.o) as in accordance with statistical analysis, using analysis of variance one-way (ANOVA) followed by a post hoc by Turkey's test for multiple comparisons and DPZ showed a significance of *$p <0.01$, (n=15). Values are mean ± SEM (n=15).

Figure 4. Marble-burying Test. To examine this premise, we studied the effect of the representative of Val, Valep and DPZ on burying behavior. As expected, Control exhibited significant decrease in the marble burying behavior. However, Val requested an increase of marble (50, 100, and 200, Valep 12 and DPZ 12 mg/kg BW). This data was evaluated using the analysis of variance one-way (ANOVA) followed by a post hoc by *Turkey's* test for multiple comparisons *$p< 0.01$. Values are mean ± SEM (n=15).

Figure 5. Effect of Hole-Board Test. The effect of Val, Valep and DPZ in board-hole test is shown in Figure 4. Promoted a significant increase in the number of head-dipping behavior was shown as *$p<0.01$, this data was evaluated using the analysis of variance one-way (ANOVA) followed by a post hoc by Turkey's test for multiple comparisons *$p< 0.01$. Values are mean ± SEM (n=15).

Figure 6A. Elevated plus-maze Test. In the elevated plus-maze at doses of 50, 100 and 200 mg / kg, and 12 mg/kg, promoted a significant difference compared with the control group and similar to the effect of DPZ in time spent in open arms. This data was evaluated using the analysis of variance one-way (ANOVA) followed by a post hoc by Turkey's test for multiple comparisons *$p< 0.01$. Values are mean ± SEM (n=15).

Figure 6B: Elevated plus-maze Test. Effect of Val and Valep at doses of 50, 100, 200 and 12 mg/kg BW respectively , was evaluated in relation to the control and DPZ. The data show a significant presence in the closed arm of the control group. Analysis of variance (ANOVA) with post hoc Turkey's test for multiple comparisons *$p <0.01$. Values are mean ± SEM (n=15) animals.

DISCUSSION

Herbal preparations are extremely common in Brazil, as old stories point to the effect of multiple plant diseases and increased use of herbal products for self-medication in Brazil has grown exponentially. The material used was obtained by us at random among the various extracts sold in the city of Recife Brazil[18]. The data obtained from the Val in behavioral tests show an anxiolytic and hypnotic effect at doses of 50, 100 and 200 mg/kg and Valep 12 mg/kg, i.p. in behavioral tests (Marble-burying test, Hole-Board Test, Elevated plus-maze and Sodium Pentobarbital) showed an anxiolytic and hypnotic effect through the tests listed above ($p< 0.01$). However, the Val and Valep did not promote the protection of animals in convulsions induced by Pentylenetetrazol (55 mg/kg). These data are consistent with literature [13, 15-17]. The compounds present in the root of Val are involved in its pharmacological action, especially Valerenic acids and Valeprotriates, which promote the inhibition of the degradation of γ-aminobutyric acid [18-20]. Our results showed strong implications for the general use of Valeriana officinalis as an anxiolytic and hypnotic in several behavioral tests. One of the many questions asked by these implications relates to the ability of valerian in the treatment of anxiety in humans in relation to traditional anxiolytics, and cross-tolerance to valerian

might occur in those who have become tolerant to the effects of benzodiazepines[12]. Based on these results we can suggest the existence of a hypnotic and anxiolytic effects of Valerian extract root (Val), and that valerian may be an effective alternative as a natural anxiolytic, which often can produce aversive side effects such as nausea, tremor, and drug addiction[21]. The physiological mechanisms of the action of Valerian on the Central Nervous System are increasingly well established. These effects are probably involved in the action of GABA potentiating or a direct action of Valep and Val at the site of the receptor or in the region of action of benzodiazepines the same receptor. The valepotriates is one of the main compounds of Valeriana officinalis, and their action has been demonstrated in the CNS. Our findings have strong implications for the general use of valerian as an anxiolytic in several behavioral tests[6, 7, 8, 19, 20]. Val and Valep were more effective by intraperitoneal route than the oral (data not shown).

CONCLUSION

The data obtained by us showed no anticonvulsant effect, unlike the literature. However, the data suggest the use of the Valeriana officinalis hypnotic and anxiolytic.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to CNPq and Capes (National Council for Research, Coordination of Improvement of Higher Education Personnel respectively) and to the Federal University of Pernambuco for their support.

REFERENCES

1. Albrecht, M., Berger, W. Zeits Allegmeinmed. Psychopharmaceuticals and safety in traffic 1995; 71: 1215-1221.
2. Brown, D. J. Herbal Prescriptions for Better Health, pp. 173-178, Prima Publishing, Rocklin, CA. 1996.
3. Gao, X. Q., Björk, L. Fitoterapia. Valerenic acid derivatives and valepotriates among individuals, varieties and species of Valeriana 2000; 71: 19-24.
4. Benke, D., Barberis, A., Kopp, S., Altmann, K. H., Schubiger, M., Vogt, K. E., Rudolph, U., Möhler, H. GABA A receptors as in vivo substrate for the anxiolytic action of valerenic acid, a major constituent of valerian root extracts. Neuropharmacology. 2009; 56; 174-1781.
5. Newall, C. A., Anderson, L. A., Phillipson, J. D. Herbal Medicines: A Guide for Health-Care Professionals, pp. 260. The Pharmaceutical Press, London. 1996
6. Neuhaus, W., Trauner, G., Gruber, D., Oelzant, S., Klepal, W., Kopp, B., Noe, C. R. Biomedically relevant chemical constituents of *Valeriana officinalis*. Planta. Med. 2008; 74: 1338-1344.
7. Khom, S., Baburin, I., Timin, E., Hohaus, A., Trauner, G., Kopp, B., Hering, S. Valerenic acid potentiates and inhibits $GABA_A$ receptors: Molecular mechanism and subunit specificity. Neuropharmacology . 2007; 53 178-187.
8. Neuhaus, W., Trauner, G., Gruber, D., Oelzant, S., Klepal, W., Kopp, B., Noe, C. R. Biomedically relevant chemical constituents of *Valeriana officinalis*. Planta. Med. 2008; 74: 1338-1344.
9. Dressing H, Köhler S, Müller WE. Improvement of sleep quality with a high-dose valerian/lemon balm preparation: A placebo-controlled double-blind study. Psychopharmakotherapie, v. 6, p. 32–40, 1996.
10. Dietz, B. M., Mahady, G. B., Pauli, G. F., Farnwsworth, N. R. Valerian extracts and valerenic acid are partial agonists of the 5HT5a receptor. Mol. Brain. Res. 2005; 138, 191–197.
11. Kohnen, R., Oswald, W. D. The effects of valerian, propranolol, and their combination on activation, performance and mood of healthy volunteers under social stress conditions. Pharmacopsychiatry. 1988; 21: 447-448.
12. Mennini, T., Bernasconi, P. In vitro study on the interaction of extracts and pure compounds from Valeriana officinalis roots with GABA, benzodiazepine and barbiturate receptors. Fitoterapia. 1993; 64: 291-300.
13. Lister RG. The use of a plus-maze to measure anxiety in the mouse Psychopharmacology. 1987; 92: 180-185.
14. Harbone, J. B. Phytochemical methods: a guide to modern techniques of plant analysis. Phytochemical methods. 3º ed. London: Chapman & Hall.
15. Speroni, E., Minghetti, A. Neuropharmacological activity of extracts from *Passiflora incarnata.*Planta Med. 1988; 54:488-491.
16. Njung'e, K., Handley, S. L. Evaluation of marble-burying behavior as a model of anxiety Pharmacol. Biochem. Behav. 1991; 38(1): 63-67.
17. Treit, D., Pinel, J. P., Fibiger, H. C. conditioned defensive burying: a new paradigm for the study of anxiolytic agents. Pharmacol.Pharmacol. Biochem. Behav. 1981; 15: 619-626.
18. Bos, R., Woerdenbag, H. J., van Putten, F. M., Hendriks, H., Scheffer, J. J. Seasonal variation of the essential oil, valerenic acid and derivatives, and velopotriates in Valeriana officinalis roots and rhizomes, and the selection of plants suitable for phytomedicines. Planta. Med. 1998; 64: 143-147.
19. Benke, D., Barberis, A., Kopp, S., Altmann, K. H., Schubiger, M., Vogt, K. E., Rudolph, U., Möhler, H. GABAA receptors as in vivo substrate for the anxiolytic action of valerenic acid, a major constituent of valerian root extracts.Neuropharmacology. 2009; 56: 174-181.
20. Trauner, G., Khom, S., Baburin, I., Benedek, B., Hering, S., Kopp, B. Modulation of GABAA receptors by valerian extracts is related to the content of valerenic acid. Planta. Med. 2008; 74: 19-24.
21. Stewart, S. H., Westra, H. A., Thompson, C. E., Conrad, B. E. Introduction to the Special Issue on Interoceptive Exposure in the Treatment of Anxiety and Related Disorders: Novel Applications and Mechanisms of Action.Cognit. Therapy. Res. 2000; 24, 67-85.
22. Rezvani, M. E., Roohbakhsh, A., Allahtavakoli, M., Shamsizadeh, A. Anticonvulsant effect of aqueous extract of *Valeriana officinalis* in amygdala-kindled rats: Possible involvement of adenosine. J. Ethnopharmacol. 2010; 127: 313-318.

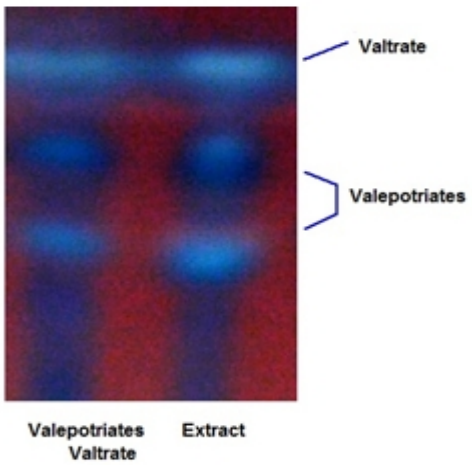

Figure 1. Phytochemical analysis showing the two main compounds (Valtrate and Valepotriates).

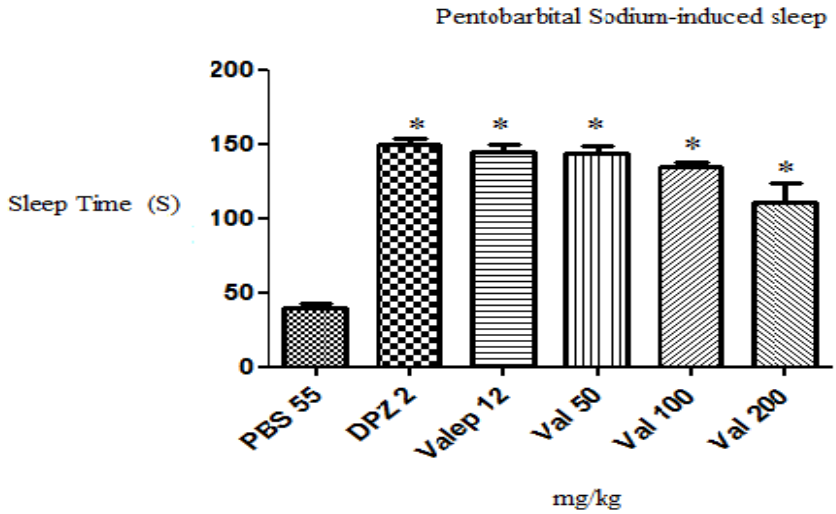

Figure 2 Sodium Pentobarbital-induced sleeps.
The effect of Pentobarbital Sodium-induced sleep is shown in Fig 4. The values, up to 50, 100 and 200 mg/kg of Val, as well as Valepar and DPZ also showed a significant effect were significantly different from the Control group * $p<0.01$. Values are mean ± SEM (n=15).

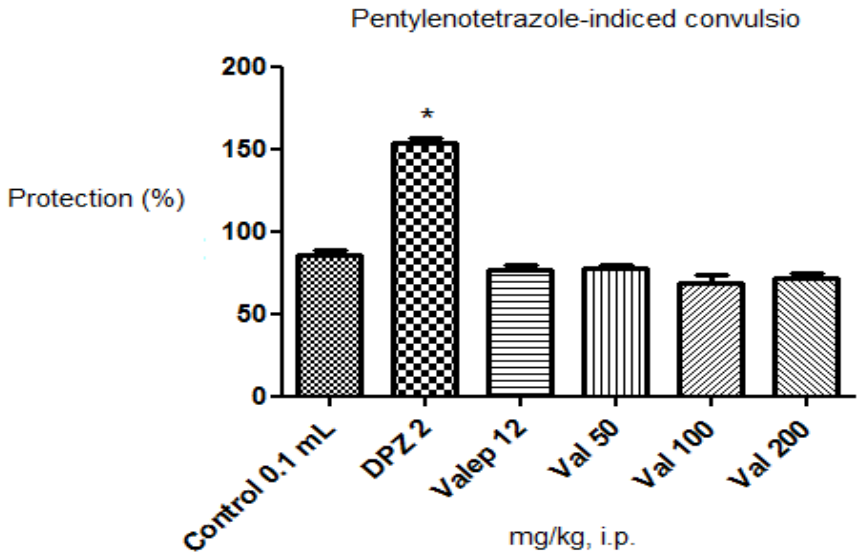

Figure 3. Pentylenotetrazole-induced convulsion Test.
Val and Valep non-inhibited generalized seizures induced by PTZ (55 mg/kg, i.p) as in accordance with statistical analysis, using analysis of variance one-way (ANOVA) followed by a post hoc by Turkey's test for multiple comparisons and DPZ showed a significance of *$p<0.01$, (n=15). Values are mean ± SEM (n=15).

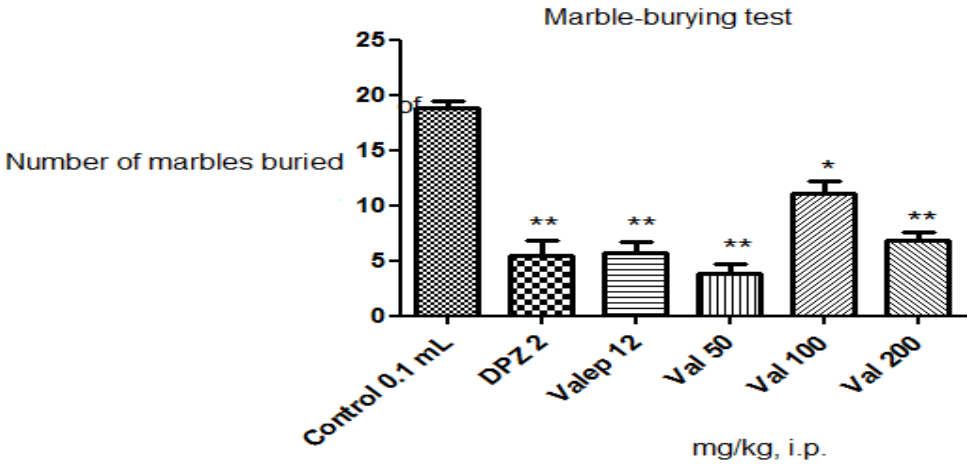

Figure 4. Marble-burying Test.

To examine this premise, we studied the effect of the representative of Val, Valep and DPZ on burying behavior. As expected, Control exhibited significant decrease in the marble burying behavior. However, Val requested an increase of marble (50, 100, and 200, Valep 12 and DPZ 12 mg/kg BW). This data was evaluated using the analysis of variance one-way (ANOVA) followed by a post hoc by Turkey's test for multiple comparisons *p< 0.01. Values are mean ± SEM (n=15).

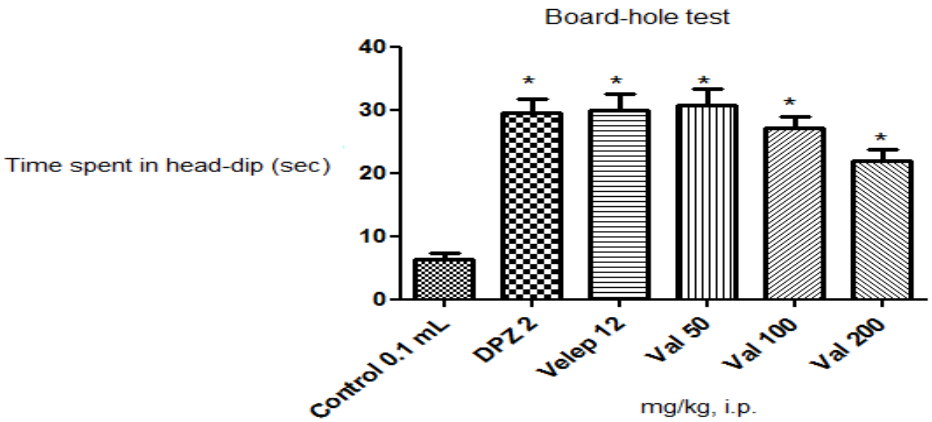

Figure 5. Effect of Hole-Board Test.

The effect of Val, Valep and DPZ in board-hole test is shown in Figure 4. Promoted a significant increase in the number of head-dipping behavior was shown as *p<0.01. This data was evaluated using the analysis of variance one-way (ANOVA) followed by a post hoc by Turkey's test for multiple comparisons *p< 0.01. Values are mean ± SEM (n=15).

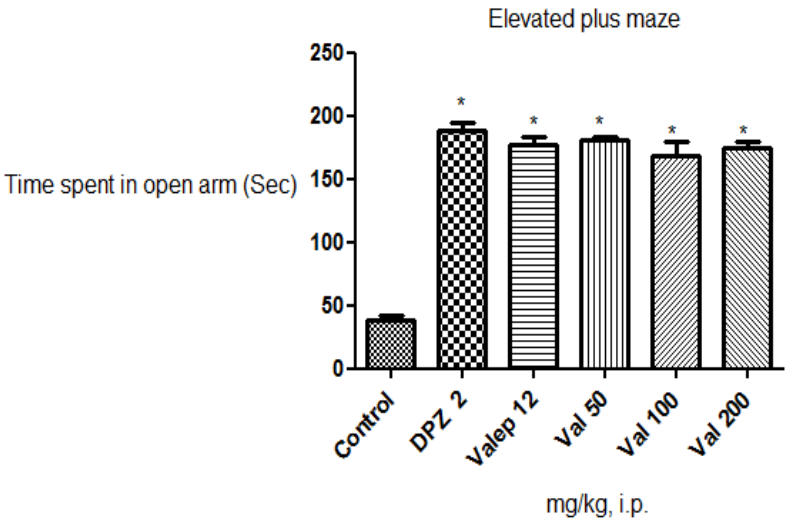

Figure 6A. Elevated plus-maze Test.

In the elevated plus-maze at doses of 50, 100 and 200 mg / kg, and 12 mg/kg, promoted a significant difference compared with the control group and similar to the effect of DPZ in time spent in open arms. This data was evaluated using the analysis of variance one-way (ANOVA) followed by a post hoc by Turkey's test for multiple comparisons *p< 0.01. Values are mean ± SEM (n=15).

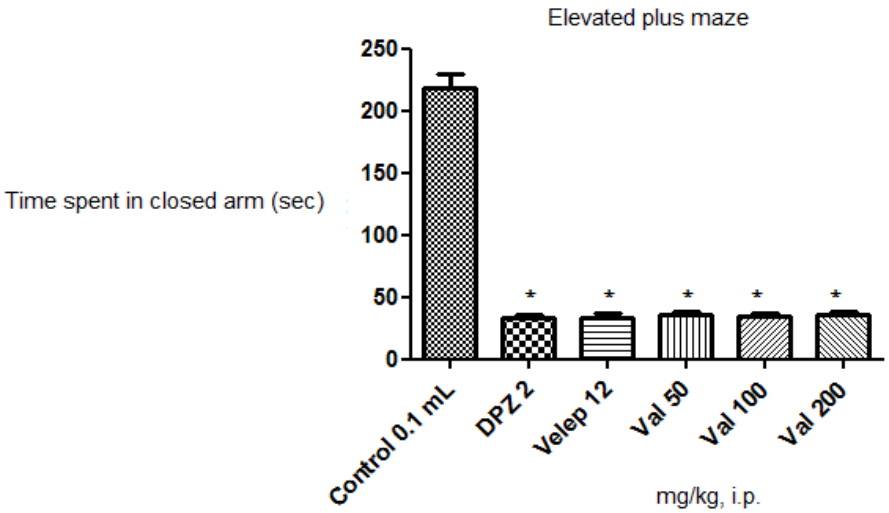

Figure 6B: Elevated plus-maze Test.

Effect of Val and Valep at doses of 50, 100, 200 and 12 mg/kg BW respectively, was evaluated in relation to the control and DPZ. The data show a significant presence in the closed arm of the control group. Analysis of variance (ANOVA) with post hoc Turkey's test for multiple comparisons *p <0.01. Values are mean ± SEM (n=15) animals.

Source of support: Nil, Conflict of interest: None Declared