Experimental evaluation of anxiolytic activity in mice using Vacha Churanam

Anamika P K¹, Sulaiman Mohammed Alnasser², Abdul Khayum K³

¹Department of Pharmacology, CL Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India
²Department of Pharmacology and Toxicology, Unaizah College of Pharmacy, Qassim University, Saudi Arabia
³Department of Pharmacology, PSG College of Pharmacy, Coimbatore, Tamil Nadu, India

Article History:
Received on: 14 Oct 2020
Revised on: 15 Nov 2020
Accepted on: 17 Nov 2020

Keywords:
Hole board, Morris water maze, Dopamine, Serotonin, Superoxide dismutase, Anxiety

ABSTRACT

Vacha Churanam is an Ayurvedic product. It is commonly given traditionally for memory enhancement, antidepressants, anxiolytic and anti-stress. However, there is no scientific evidence for its anxiolytic activity. Therefore the current study is aimed to validate its anxiolytic activity. Kept mice persuaded the anxiety in the room maintained with a temperature of 4°C for two h per day. The Vacha Churanam has administrated doses of 200 and 400 mg/kg, p.o for 21 days. The behavioural parameters such as elevated zero maze, light-dark test, Morris water maze, actophotometer, hole board were produced significant activity with Vacha Churanam. The neurotransmitters measures such as dopamine, serotonin and acetylcholine esterase measures were also increased in Vacha Churanam treated mice when matched with negative control group mice. The antioxidant enzyme superoxide dismutase was increased, and lipid peroxidase level was diminished in Vacha Churanam treated mice. It supports the anxiolytic action of Vacha Churanam through an antioxidant mechanism. The Anxiolytic result of Vacha Churanam is associated with essential constituents such as flavonoids, phenolic and steroids.

INTRODUCTION

Anxiety is a feeling of unease and fear, typically general and unclear overreaction. It is related to restlessness, muscular tension, fatigue and problems in concentration (Davison, 2008). As per the World Health Organization (WHO), one in 13 people affected globally with anxiety. The anxiety disorders stand worldwide caused mental disorders with phobia and chief depressive illness (WHO reports, 2017). There are many medications including Escitalopram, Fluvoxamine, Sertraline, Duloxetine, Alprazolam, Chlordiazepoxide, Diazepam, Oxazepam, Lorazepam, Clomipramine, Imipramine, Phenelzine, Hydroxyzine, Buspirone can be utilised currently to the treatment of anxiety (Murrough et al., 2015). The difficulty in the synthetic drugs are imperfectly tolerated or assemble substantial undesirable side effects (WHO reports). The widely used medicine to treat anxiety is benzodiazepines as they deliver fast and significant relief however the problem with the benzodiazepines is harm cognitive function while using them, and long-standing practice could cause permanent memory deficiency (Guina and Merrill, 2018). Most people wishing substitute treatment including for anxiety including meditation practice, Ayurveda, Siddha and homeopathy, play a part in mental, behavioural therapy, decreasing stress and workout (Qureshi
Vacha Churanam is an ayurvedic product. It is commonly used traditional product for the memory enhancement, antidepressants, anxiety and anti-stress etc., (Sharma et al., 2020). However, there is no scientific confirmation for its anxiety and anti-stress activity. Therefore the current study is aimed to validate its anxiety and anti-stress activity.

MATERIALS AND METHODS

Materials

The marketed ayurvedic product Vacha Churanam was purchased from online shopping website Ayu svastha. Diazepam was purchased from Sigma Aldrich, USA. All other reagents and chemicals were used under analytical grade.

Preliminary phytochemical analysis

Vacha Churanam was mixed with distilled water and subjected to preliminary phytochemical screening for the confirmation of phytoconstituents such as alkaloids, carbohydrates, protein, steroids, phenols, tannins and flavonoids (Gopalasatheeskumar et al., 2017; Trease and Evans, 1983).

Experimental animals

Albino mice (22-30g body weight) were attained from the animal household of C.L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai-97. All experimentation were supported to the guidelines for attention and usage of experimental animals and approved by the Committee for Control and Supervision of Experiments on Animals (CPCSEA). The experimental ethical was permitted by the Institutional Animal Ethical Committee (IAEC). IAEC reference no: IAEC/LI/01/CLBMCP/2017.

Acute toxicity studies

The OECD guideline 423 was followed for the acute toxicity to the fixing of experimental dose. In acute toxicity study, 2000 mg/kg, p.o different dose of Vacha Churanam was given for three animal containing groups. Then mice have withheld the feed for 3-4 h and monitored carefully for the marks of toxicity for 15 successive days (OECD-423). (OECD/OCDE, 2001)

Grouping of animals

Totally 30 mice were separated into five groups and each for six animals. Group I: Control mice were only received normal saline. Group II: Negative Control mice were received normal saline. Group III: Standard mice served with Diazepam 5mg/kg, i.p. Group IV: Vacha Churanam low dose mice served with Vacha Churanam 200mg/kg, p.o. Group V: Vacha Churanam high dose mice served with Vacha Churanam 400mg/kg, p.o.

Induction of anxiety

The negative control, standard treatment and Vacha Churanam treatment groups, were preserved in a room maintained with the temperature of 4°C for two h per day for the generation of stress followed by anxiety. The normal control group was only received normal saline for the entire study. The treatments for the respective groups were for 21 days. The final dose was given 60 min earlier to behavioural testing (Lian et al., 2019).

Estimation of behavioural parameters

The behavioural examinations were conducted on the 21st day of the experiment. The behavioural examinations including locomotor test (Actophotometer) (Bhosale et al., 2011). Elevated zero maze (Singh et al., 2007), Dark-light compartment (Takao and Miyakawa, 2006) and Marble burying tests (Thomas et al., 2009) were conducted by standard procedure.

Estimation of neurotransmitter level

After the experimental period, all group mice were humanly killed by cervical decapitation and Brain were isolated. The isolated brain was homogenate with HCl – Butanol solution and pH 7.4 solutions and allowed 10 min for centrifugation at 10000 rpm (Boopathi et al., 2020). The obtained supernatant undergoes the valuation of neurotransmitter including Dopamine, Serotonin and Acetylcholinesterase enzyme using standard procedure followed by literature (Brownlee and Spriggs, 1965).

Estimation of antioxidants levels

On the completion of the investigational period, all group mice were humanly killed by cervical decapitation for isolation of Brain. The isolated brain was homogenate with ice-cold TCA pH 7.4 solutions and allowed 10 min for centrifugation at 10000 rpm (Gopalasatheeskumar et al., 2020). The obtained supernatant undergoes the valuation of in vivo antioxidants levels including Lipid peroxidation (LPO) and Superoxide dismutase (SOD) as per standard process followed by literature (Yuvaraja et al., 2020; Ohkawa et al., 1979).

Histopathological study

On the final day experiment, the brains of all group animals were carefully detached without damage. Then the isolated brains were rinsed using ice-cold saline solution and stable in 10% neutral buffered formalin. The five μ sections of tissues were stained using Mayer’s Haematoxylin Eosin dye.
Statistical analysis

The statistical analysis was carried by one-way analysis of variance (ANOVA) monitored by Dunnett’s test. P values <0.05 (95% confidence limit) was measured statistically significant, by Software Graph pad Prism 6.0.

RESULTS

Preliminary phytochemical Qualitative analysis

The preliminary phytochemical examination endorses the numerous phytoconstituents including alkaloids, carbohydrates, Glycosides, Saponins, Anthraquinones, protein, steroids, phenols, tannins and flavonoids existence in Vacha Churanam.

Acute oral toxicity studies

A single dose (2000 mg/kg p.o) of the Vacha Churanam did not persuade any sign of toxicity. It confirms the safe dose of Vacha Churanam at 2000 mg/kg. Therefore we fixed the 1/10th (200mg/kg) and 1/5th (400 mg/kg) dose for further experimentation.

Effect of Vacha Churanam on Behavioral parameters

Our finding in elevated zero maze test shown that negative control mice were decreased in percentage time consumed within open arm and percentage passes within an open arm. In the light-dark test, negative control mice were decreased the time consumed in the light compartment. Similarly, in the whole, board test, negative control mice have decreased the sum of line crossing, head dipping and amount of rearing. These decreased levels were enhanced significantly improved in after 21 days Vacha Churanam (200 mg/kg and 400 mg/kg) and standard diazepam administration. However, negative control mice were buried more amount of marbles in the marble-burying experiment, higher escape latency in the Morris water maze experiment. They augmented the locomotors activity in Actophotometer test. In Vacha Churanam (200 mg/kg and 400 mg/kg) and diazepam treated mice groups were reported signifi cantly lowered (Figure 1).

From Figure 1, A: Elevated zero maze test, B: Light-dark test, C: Marbleburying test, D: Morris water maze test, E: Locomotors test (Actophoto meter)and F: Hole board test. (Data is expressed as Mean ± SEM; n=6, One way ANOVA followed by Dunnett’s test; ns = non-significant; Compared with disease control;* p<0.05, ** p<0.01, *** p<0.001).

Effect of Vacha Churanam on neurotransmitters

Three neurotransmitter endogenous chemical messengers including Acetylcholine esterase, Serotonin and Dopamine measures were significantly diminished in negative control mice when compared with normal control mice. Interestingly, both Vacha Churanam (200 mg/kg and 400 mg/kg) and standard diazepam treated mice were significantly increased (Table 1).

Effect of Vacha Churanam on antioxidants level

The antioxidant levels, including SOD and LPO, were estimated. The brain SOD measures in negative control mice were significantly declined, while LPO measures were significantly augmented when both of them were matched with normal control. In contrast, Vacha Churanam (200 mg/kg and 400 mg/kg) and standard diazepam treated mice showed that antioxidant measures were significantly normalised (Table 2).

Histopathological study of Brain

In the negative control mice, the density of neuronal cells and disturbance of neuronal cells were decreased. In the other hand, groups treated with Vacha Churanam (200 mg/kg and 400 mg/kg) and standard diazepam exhibited improved neuronal configurations (Figure 2).

From Figure 2, A: Normal Control, B:Negative Control, C: Standard, D: Vacha churna 200mg/kg and E: Vacha churna400mg/kg. Mayer’s Haematoxylin Eosin stain was used for staining.

DISCUSSION

Benzodiazepines are expansively used anxiolytic synthetic drugs. But commonly Benzodiazepines associated with unwanted side effects so that alternative treatments are needed. Medicinal herbs and herbal therapy like Siddha, Ayurveda and Unani are an excellent foundation to find new remedies to treat these disorders. In the study for a substitute, more specific and perhaps cost-free therapy, researchers are focusing on investigating natural anxiolytic drugs. The Vacha Churanam is an Ayurvedic product marketed for controlling the anxiety. In the current research, the anxiolytic action of 200 mg/kg and 400 mg/kg of Vacha Churanam on the Elevated zero maze, light-dark test, actophometer, morris water maze, hole board were almost same comparable with 4 mg/kg of diazepam. The neurotransmitters, including dopamine, serotonin and acetylcholine esterase measures were also significantly elevated because of anxiolytic activity. The elevated measures of SOD, deteriorated measures of LPO in Vacha Churanam confirm the antioxidant action of anxiolytic activity. These observa-
Figure 1: Effect of Vacha churna on Behavioral parameters

Table 1: Effect of Vacha Churanam on neurotransmitters

| S. No | Group            | Acetylcholine Esterase (ng/mg wet tissue) | Dopamine (ng/mg wet tissue) | Serotonin (ng/mg wet tissue) |
|-------|------------------|-----------------------------------------|----------------------------|------------------------------|
| 1     | Normal control   | 25.63±0.57                              | 430.23±20.34               | 243.34±5.6                   |
| 2     | Negative Control | 12.34±0.67                              | 308.45±2.45                | 181.33±5.34                  |
| 3     | Standard         | 23.34±0.12**                            | 400.23±3.2**               | 235.55±5.32**                |
| 4     | Vacha Churanam 200mg/kg | 15.34±0.2*                            | 239.47±2.55               | 203±2.72ns                   |
| 5     | Vacha Churanam 400mg/kg | 20.98±0.11**                           | 381.45±4.34*              | 225.34±2.89*                 |

No. of Animals 6, Data were analysed as Mean ± SEM, One way ANOVA followed by Dunnett’s test, All groups were matched with negative control, *p<0.05, **p<0.01, ***p< 0.001
Table 2: Effect of Vacha Churanam on antioxidants

| S. No | Group                        | SOD (Units/mg wet tissue) | LPO (Units/mg wet tissue) |
|-------|------------------------------|----------------------------|----------------------------|
| 1     | Normal control               | 9.32 ± 0.4                 | 70.1 ± 0.3                 |
| 2     | Negative Control             | 4.1 ± 0.25                 | 109.3 ± 0.23               |
| 3     | Standard                     | 9.02 ± 0.41**              | 72.34 ± 1.3**              |
| 4     | Vacha Churanam 200mg/kg     | 5.99 ± 0.3***              | 95.45 ± 1.23***           |
| 5     | Vacha Churanam 400mg/kg     | 7.187 ± 0.21*              | 80.23 ± 1.32*             |

No. of Animals 6, Data were analysed as Mean ± SEM, One way ANOVA followed by Dunnett’s test, All groups were matched with negative control, *p<0.05, **p<0.01, ***p<0.001
tions indicate that Vacha Churanam exerts an anxiolytic activity. The anxiolytic effect of Vacha Churanam is might be associated with essential constituents, including flavonoids, steroids and phenolic composites (Farzaei et al., 2016). The literature review has suggested that many scientific trials have been stated that Vacha Churanam exhibited many properties in the central nervous system (CNS) disorders. Vacha Churanam is also intended for the managing of CNS disorders. Vacha Churanam might be appreciated in the controlling of neurodegenerative diseases including Alzheimer’s disease, memory-improving property and anti-cholinesterase activity.

CONCLUSION

In conclusion, the current research confirms the anxiolytic activity of Vacha Churanam against cold-induced stress on mice. The effect of Vacha Churanam might be the manifestation of phytochemicals such as flavonoids, steroids and phenolic. Further molecular pharmacological and chemical researches are required to expose the exact mechanisms of anxiolytic action and to separate the active phytochemicals.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES

Bhosale, U. A., Pophale, P. D., Somani, R. S., Yegnarayan, R., Zambare, M. R. 2011. Study of central nervous system depressant and behavioral activity of an ethanol extract of Achyranthes aspera (Agadha) in different animal models. International Journal of Applied and Basic Medical Research, 1(2):104–108.

Boopathi, T., Gopalasatheeskumar, K., Ariharasivakumar, G. 2020. Exploration of antiparkinson activity of aqueous extract of Barleria prionitis with antioxidant potential in MPTP and rotenone Induced Parkinson rat models. International Journal of Biology, Pharmacy and Allied Sciences, 6(2):79–91.

Guina, J., Merrill, B. 2018. Benzodiazepines I: Upping the Care on Downers: The Evidence of Risks, Benefits and Alternatives. Journal of Clinical Medicine, 7(2):17–17.

Qureshi, N. A., Al-Bedah 2013. Mood disorders and complementary and alternative medicine: a literature review. Neuropsychiatric Disease and Treatment, 9:639–658.

OECD/OCDE 2001. OECD guideline for testing of chemicals. Acute Oral Toxicity – Acute Toxic Class Method. Pg. 1-14.

Ohkawa, H., Ohishi, N., Yagi, K. 1979. Assay for lipid peroxides in animal tissues by the thiobarbituric acid reaction. Analytical Biochemistry, 95(2):351–358.

Singh, K., Bishnoi, M., Kulkarni, S. K. 2007. Elevated Zero-maze: A paradigm to evaluate anti-anxiety
effects of drugs. *Methods and Findings in Experimental and Clinical Pharmacology*, 29(5):343–348.

Takao, K., Miyakawa, T. 2006. Light/dark Transition Test for Mice. *Journal of Visualized Experiments*, 13(1):104–104.

Thomas, A., Burant, A., Bui, N., Graham, D., Yuval-Paylor, L. A., Paylor, R. 2009. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology*, 204(2):361–373.

Trease, G. E., Evans, W. C. 1983. *Textbook of Pharmacognosy*. 12th Edition. pages 343–383, London. Balliese Tindall and Company Publisher.

WHO reports 2017. Depression and Other Common Mental Disorders. Global Health Estimates. World Health Organization.

Yuvaraja, K. R., Santhiagu, A., Jasemine, S., Kumar, K. G. 2020. Hepatoprotective activity of Chloroform and Ethyl acetate extract of Dipteracanthus patulus against Paracetamol induced Hepatotoxicity in rats through Antioxidant mechanism. *Research Journal of Pharmacy and Technology*, 13(1):203–208.