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EVALUATION OF ACUTE AND CHRONIC TOXICITY, COGNITION IN ADULT ZEBRAFISH WITH VASICINE; A PROSPECTIVE COGNITION ENHANCER IN NEUROLOGICAL DISORDERS

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ABSTRACT

Traditionally the herb *Justicia adhatoda* has been used widely as antitussives, antispasmodic, and abortifacient. But quantitative neurotoxicity studies of Vasicine are scarcely explored. Therefore establishing a toxicity dosage would enable safe therapeutic prescription. There are number of studies which have performed toxicity with adhatoda extract, however isolated Vasicine toxicity profile screening in zebrafish has scarcely been discussed. A whole animal toxicity screen, including cognition, organ pathology and anatomy in orally administered fish would allow screening all vital organs, and behavior as an efficient toxicity study set up. Moreover orally administered vasicine would be subjected to the gastric environment and secondary metabolism allowing a more close comparison to oral route of dosage. Adult zebrafish were treated with different concentrations of vasicine orally and were periodically accessed for a 14 day acute period and 30 day chronic period. Both organ pathology and cognitive studies showed no toxicity on oral administration of a total 18.5 ngfor 30 days. It can be said that vasicine is safe at nanogram quantity for a zebrafish when administered orally. This could translate into milligrams quantity in mammals or micrograms in larger mammals. Hepatic metabolism could render the molecule safer and more efficient. By therapeutic index standards the dosages falls within a wide range of therapeutic potential before inducing toxicity. Since Vasicine with our preliminary docking studies have proven hypothetically to be a prospective molecule in inhibiting cAMP specific phosphodiesterase enzyme (PDE7B) , therapeutic dosages of Vasicine could be promising as a potential therapy in cognitive enhancement for neurological disorders such as Alzheimer's and Parkinson disease, multiple sclerosis, etc.

KEYWORDS: **Vasicine, Toxicity, Zebrafish, Pathology, Cognitive Screen, Oral Administration, Neurological Disorders**

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INTRODUCTION

Zebrafish is now a standard model of choice for speed; economy, whole animal live imaging with a genome sequenced. Also pharmacokinetics and dynamics can be evaluated in this model. A number of high through put state of art techniques can be performed in Zebrafish in the areas of forward and reverse genetics, expression profiling and Transgenesis.[1-2] A number of transgenics with valuable drug targets are available in the market. There is a strong evidence of linking target to function and numerous studies have proven this in Zebrafish. Moreover the ability to combine multiple discovery steps into fewer numbers of steps allows integration of drug discovery. Zebrafish is therefore the best vertebrate model for accelerate integrated discovery with inclusion of developmental screening.[3] All parts of the plant *Justicia adhatoda* have been used for various kinds of treatments such as in infections as in Malaria and typhus fever, in Inflammation as in rheumatism, Bronchodilator as in cold, and also in Veneral diseases. Therefore different parts of the plant can be used for different treatments and it is likely that the compounds from *Justicia adhatoda* have multiple sites of action. Several other alkaloids such as Vasicine, Vasicinol, Adhatodine, Adhatonine, Adhvasinone, Anisotine and Hydroxypeganine are found in the plant leaf.[4] Both DPPAH and Ferric Oxide reduction Antioxidant assays have confirmed strong antioxidant activity. Therefore the mode of action of Vasicine could be attributed to both antioxidant activity coupled with other physiological activities.[5] Antioxidant activity has been further reinforced by protective activity in varying dose study of tobacco as a toxin.[6] Further the plant extract has proven to directly interact with influenza virus and prevent it from binding with cell targets.[7] The plant extract accelerated epithelization and contributed to quicker wound healing in rat studies.[8] Studies on Pharmacognosy and chelation properties of heavy metals proved having a heterogenous type of phytochemicals with proven effect on direct chelation and deactivation of heavy metal toxins such as mercury.[9,10] In Guinea Pigs the plant has proven mechanisms, including stimulation of β-adrenergic receptors and inhibition of histamine H1 receptors.[11] Both thrombopoeitic and activity of volatile compounds again tuberculosis have also been observed[12]. Also chronic disorders such as diabetes and epilepsy have showed marked improvement on treatment with the plant.[13,14] However studies on establishing a toxicity dosage, kinetics and mode of administration have not been standardized. Therefore here we standardize the dosage in zebrafish model which could be translated to larger animal studies for further clinical development.

MATERIALS AND METHODS

Housing

Wild type Groups of 12 adult male fishes with similar weight were housed at light dark cycle of 14/10; water temperature of 26^0C in 3 L water tanks with continuous aeration was maintained. Fishes were fed twice with regular fish feed and regularly screened for abnormalities. Three groups maintained were control group fed with placebo as applicable and two other groups were evaluated for acute and chronic toxicity.

Oral Administration of Vasicine

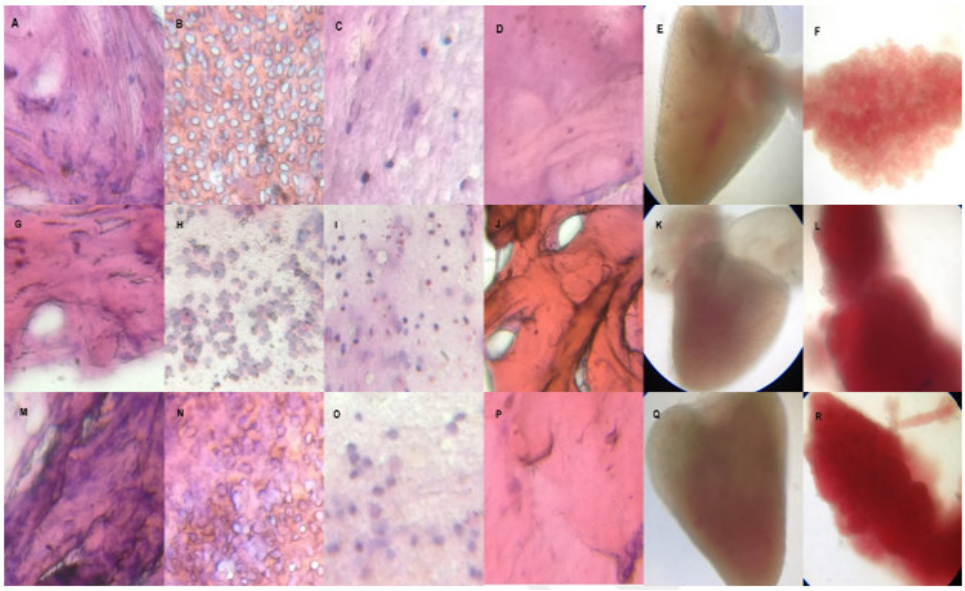
Commercial Vasicine from Natural Remedies, Bangalore was obtained and purity showed 95% purity by HPLC. 7.5 mg Vasicine was dissolved in 10 ml of 10% DMSO to make a solution and formulated to a solid feed. Fishes were fed once a day with formulated solid feed containing 0.61ng per day of Vasicine totaling to 8.54ng for 14 days and 18.5 ng vasicine for 30 days. Fishes were also fed regular feed twice a day.

Screening for Toxicity

Fish were euthanized after cold temperature anesthesia below 15^0 C and Liver, Heart, Brain and skeletal muscle tissue were removed and smear stained with Haematoxylin and Eosin. For behavioral and motor toxicity swim motion test and dive tank test were performed. Time taken to swim between quadrants and time spent on either top or bottom of the tank were calculated.

RESULTS

Figure 1
Histopathology of Heart, Liver, Brain, Skeletal Muscle and Anatomy of Heart, and Liver



A-F: Control, G-L: Acute, M-R: Chronic in the order of Pathology of Heart, Liver, Brain, Skeletal Muscle, Anatomy of Heart, and Liver.

Figure 2
Histogram showing behavioral and motor response comparisons of Control, Acute and Chronic Groups

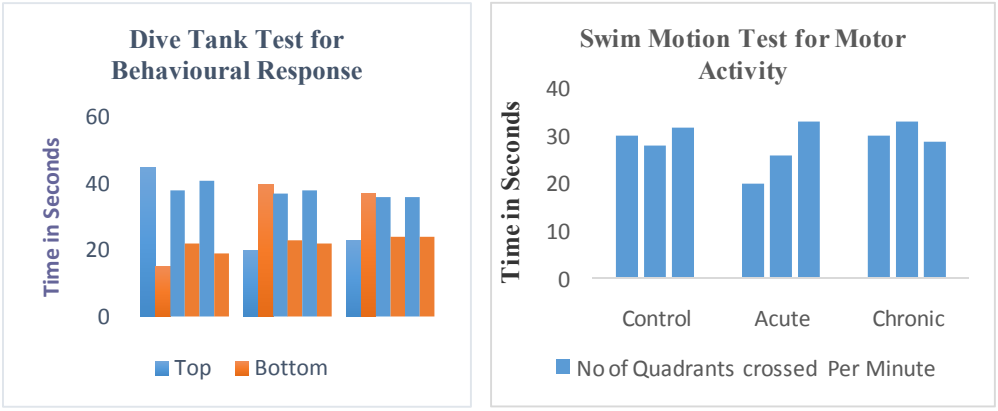


Figure 1 and 2 clearly demonstrates the results obtained from histopathology and behavioral and motor response of control, acute and chronic group animals. Normal cells are observed in control, Acute and Chronic groups showing no signs of toxicity. No inflammation, abnormal tissue architecture, apoptotic or necrotic cells were observed. This is in line with the behavioral response where no anxiety was observed.

DISCUSSION

From the results there is clear evidence of no toxicity when the fish are fed with Vasicine. However when Vasicine was administered through water diffusion into the body and hepatotoxicity was observed. It is therefore concluded that therapeutic dosage of Vasicine can be administered orally and also toxicity range varies for between oral and other routes of administration. A number of targets in Zebrafish proves that disease modeling can be done such as Alzheimer's disease, Huntington's disease. Amyotrophic lateral sclerosis, Muscular dystrophy, Thrombosis Factor VII, Cardiomyopathy, and Diabetes.[15, 16] The cholinergic, gluatamatargic, and GABAergic system in Zebrafish renders it a viable neurological disorder model for modelling Alzheimer's and Parkinson's disease. [17] Also cytopathological observation of the brain and nervous systems are clearly evident and protocols for quick screening are standardized[18, 22] Neurotoxicology models of Zebrafish are standardized using Rotenone and Paraquat[19] and end points have been well defined at behavioral, transcriptome and nerve growth levels.[20] Also both Alpha beta and Gamma Secretase targets of Alzheimer's disease have been modeled in Zebrafish, targets which have drugs in active pipeline of current pharma.[21]Antisense oligo's have been standardizing to knock down specific genes and specific symptoms also

can be modified such as in epilepsy. [23, 24] This entire set of neurological modeling can then be linked to more than ten behavioral screening domains [26] through a high through put screening system. [25] Therefore rendering end to disease modeling and high through put screening in Zebrafish. While formulations of Vasicine are being standardized and quantitative determinations of pharmaceutical developments standardized. [27, 28] There is mounting evidence of neuroprotective effects of Vasicine as through cholinesterase pathway, [29]this coupled with other N-oxides [30] and antioxidant could stimulate repair and protection against oxidative damage induced by aggregates in neuropathology. Moreover with our preliminary docking studies vasicine has been proven hypothetically to be a prospective molecule in inhibiting cAMP specific phosphodiesterase enzyme (PDE7B). Therapeutic dosages of Vasicine could bepromising as a potential therapy in cognitive enhancement for neurological disorders such as Alzheimer's and Parkinson disease, multiple sclerosis, etc. Therefore there is a strong evidence of prospective neuroprotective effect at the molecule pathway level. This has not been explored traditionally or either currently and therefore could be fully explored for neuropathological discovery targets. Further the molecule is flexible to photochemical modification and can be used in technology which involves non invasive local application;[31] whereas a number of invasive stimulators are used in neuropathology treatment, the flexibility of the molecule helps by pass invasiveness minimizing risks and saving cost. While only a few of the physiological benefits of Vasicine have been understood a detailed physiological screening would unravel the multiple benefits of Vasicine [32] exposing potential for multiple targets. This would also place the molecule highly lucrative for pharma as currently payer pressure mounts towards drugs with more than one benefit. To conclude a therapeutic potential dosage of vasicine has been established in Zebrafish and with strong evidence for neurological applications, vasicine will be a compelling candidate for clinical applications in neurological disorders.

CONCLUSION

Vasicine from *Adhatoda justica* by our preliminary docking studies have proven hypothetically to be a prospective molecule in inhibiting cAMP specific phosphodiesterase enzyme (PDE7B). Therapeutic dosages of Vasicine could be promising as a potential therapy in cognitive enhancement for neurological disorders such as Alzheimer's and Parkinson disease, multiple sclerosis, etc.

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