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Effect of early exposure of Acorus calamus on development of wistar rat off springs

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Abstract

Introduction: Acorus calamus is commonly called as sweet flag and it plays a major role in alternative medicine. The extract prepared from rhizome part posesses activity like anticonvulsant, antispasmotic and antidepressant etc. Action of phytochemicals of this extract on various systems like CNS, Gastrointestinal were analysed in adult rats. But the effects of A.calamus on developing brain in young rat offsprings have to be studied. Hence the study was undertaken with the aim, to assess the effects of early exposure of A.calamus on behavioural development of wistar rat off springs. Rat off springs were divided into two groups. On 3rd postnatal day Group 1 (n=6) was given normal saline orally. Group 2 (n=6) was given Ethanolic extract of A.calamus by orally in the dose of 600mg/kg. Both group rats off springs were observed for weight gain, ear and eye opening time. After one week of postnatal period Geotaxis test and olfactory discrimination test were done. Weight was significantly increased in group II when compared to control group. Olfactory discrimination latency time was lower in treated group. Average days of Eye and ear opening were better in study group. Ethanolic extract of A.calamus can be considered as appetite stimulant in immature rats. It may also have positive role in developing brain by promoting the maturation of nervous system.

Key words: Ethanolic extract of Acorus calamus, Behavioural Development, Geotaxis, Olfactory discrimination.

1. Background

Acorus calamus which is commonly known as Sweet flag/VACHA is a medicinal plant used for the treatment of various diseases and disorders. Sweet flag has a very long history of medicinal use in Asian herbal practice. Both Siddha and Ayurvedha mentioned Acorus calamus for its therapeutic applications. A.calamus is medicinally used for the treatment of various ailments like nervous disorders, bronchitis, chest pain, colic, cramps, diarrhea, digestive disorders, flatulence, gas, indigestion, rheumatism, sedative, cough, fever, bronchitis, inflammation, depression, tumors, hemorrhoids, skin diseases, numbness, general debility and vascular disorders [1,2]. Phytochemical analysis of rhizome part of this plant is extensively studied, the major compounds present are asarone (beta and alpha isomer), saponins, lectins, sesquiterpenoids, lignans, steroids, hydrocarbons, carbonyl compounds, alcohols, phenols, furans, and other compounds. Eight new sesquiterpenes and new norsesquiterpene named calamusins also isolated from ethanolic extract of A.calamus. Recent study supports that in vitro assay of these compounds has hepatoprotective activity [3]. Ethanolic extracts of A. calamus, especially alpha-asarone exert an anticonvulsant effect on seizures induced experimental animals [4].

In southern part of Asia at home there is routine traditional practice of administering A.calamus to normal new-borns in order to maintain good health. It has also been used in the treatment of digestive disorders and childhood colic. But still today there are no scientific

studies to support the safety and efficacy of this plant among infants. There is unanswerable question that whether A.calamus has positive effects in young developmental brain. Research supporting the influence of A.calamus in nervous system of adult rats is available but not in rat off springs. Ayurveda also recommends A.calamus for schizophrenia, alzheimer's disease and other mental disorders [4, 5]. Though the clear mechanisms are not known, it has significant CNS actions such as sedative, hypnotic, tranquilizing, and memory enhancing [6].

Developing brain of healthy new-borns need package of protein, essential fatty acids, vitamins, and minerals, which are helpful for maturation of the nervous system. In first postnatal week of rat offsprings, the early exposure of any kind of chemicals may have effect on young developing brain. Development of CNS was monitored by assessing its mile stones and early behavioural development.In rodents the sense of olfaction and balance was well developed at early post natal days. So in this study, weight gain (appetite), olfactory discrimination test, geotaxis test and mile stones assessment like eye, ear opening time were measured. Previous studies done on adult rats have proved that A.calamus can act over exitatory neurotransmitor. The positive roles of A.calamus like anticonvulsant, antidepressant are studied in matured rats, but its effect on early postnatal period has to be studied. Hence this research work was undertaken with the aim to assess the Effect of early exposure of Acorus calamus on developmental stones of Wistar rat off springs.

2. Methodology

This experimental study was conducted in the department of Physiology- Meenakshi Medical College Hospital & Research Institute after getting the Institutional Animal Ethical committee clearance (IAEC No:001/2019). Three Pregnant Wistar rats (Rattus norvegicus) were selected and relocated to the Animal House and allowed to acclimatize in current environment. Animals were maintained as per CPSEA guidelines. The rats were maintained at a room temperature of 20–22◦C, with a 12 h light/dark cycle and humidity around 60 ± 5 %. The pellets and water was provided at libitum. The adult rats were continued to monitor throughout the pregnancy period. Pregnant rats were housed individually in polypropylene cages and were allowed to raise their own litters, the litters needed for the growth and behavioural development studies alone selected randomly. The same day after birth was considered as postnatal day 0 (PN-0). Rat off springs in all three dams were observed for their suckling behaviour and health status. First three days of postnatal period was allowed for the rats and rat’s litters to adapt to postnatal period. Four litters from each cage total 12 in number were picked up for assessment and identification points were marked. They were divided into two groups

Group-1: Control group (n=6) – Exposed to normal saline.
Group -2: Study group (n=6) – Exposed to ethanolic extract of A.calamus.

Preparation of Ethanolic extract of A. calamus

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Interventional herb – Rhizome part of was A. calamus purchased from CSIR-SIDDHA institute Chennai-India. Code number of A. calamus is A27091901C.Authetication certificate for A.calamus (148-2709190) was collected from the department of Pharmacognosy in the same institute. Ethanolic extract was prepared in central Research lab of MAHER University. Rhizome powder of A. calamus was extracted with 95% ethanol for 12 hours in Soxhlet extractor. The obtained extract was concentrated using rotary vacuum evaporator at 40- 60°C. The concentrated semisolid extract was stored in refrigerator at 2-8°C till further use [7].

Administration of Ethanolic extract of A. Calamus

Study group rat litters were given ethanolic extract of A.calamus in the dosage of 600mg/kg[8] by oral route at 3^{rd} postnatal day. Small size rubber pipette was used to provide oral route in order to prevent damage to oral cavity. The following parameters were assessed. Birth weight was measured for all the rat litters.

Postnatal growth and maturation development

Pups were evaluated for weight gain on postnatal days of 0,3,6,9. Eye and ear opening day were assessed.

Geotaxis test: This behaviour was observed once daily on 7^{th} PND. The rodent is usually placed in 25^0 inclined surface in the head downwards position. The stainless grid surface allows for grip and allows for the rodent to reorient itself towards an upwards position. Scoring is done with three categories: measuring the ability to turn upwards within 60 seconds given the grade 2, attempted but failed to turn within 60 seconds given as grade 1, failure to turn within 60 seconds given as grade 0[9].

Olfactory discrimination: This test was conducted on 9^{th} and 11^{th} PNDs. The apparatus consisted of a plastic container $20x8x8cm^3$ (lxwxh), two small bins, and a clear plastic cover, which was placed over the bins and container. One end of the container contained a bin filled with clean bedding, while at the other end, there was a bin filled with home cage bedding. A $3cm^2$ area demarcated the center of the screen. A line was drawn on the screen above each bin. Each pup was placed in the centrally demarcated region on the screen, and the latency to enter the home bedding side by crossing the designed line with the front paws and head was timed. Central placement of the pup was balanced by altering the pup facing to or away the experimenter. This test reflects a nest-seeking response mediated by the olfactory system [10].

Statistical analysis

Data was presented as mean ±SD. Statistical analysis was done by using graph pad prism. Unpaired student's t test was used to compare the independent groups. $P<0.5$ was fixed as significant.

3. Results

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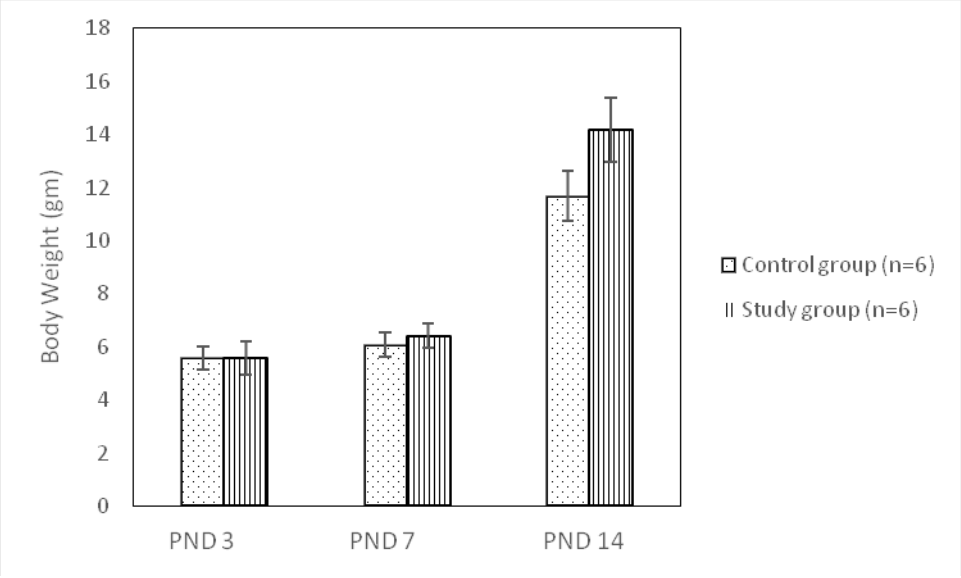
Body weight at 3rd, 7th and 14th postnatal days was measured for both groups and comparison was done. Though in first week, there was no significant difference, second week of postnatal period A.calamus exposed group showed weight gain.

Table 1: Comparison of body weight between control and study group

Body weight	**Control group (n=6)**	**Study group (n=6)**	**P value**
3rd PND	5.58±0.45	5.58±0.61	1.0
7th PND	6.08±0.45	6.42±0.45	0.2
14th PND	11.67±0.94	14.17±1.21	0.002*

P<0.5 was fixed as significant.

Graph 1: Comparison of body weight between control and study group



P<0.5 was fixed as significant.

Table **2:** Comparison of ear opening days between control and study groups

Developmental mile stones	**Control group (n=6)**	**Study group (n=6)**	**P value**
Eye opening time (days)	15.17±0.69	14.33±0.47	0.63
Ear opening time (days)	4.50±0.5	4.17±0.37	0.2

P<0.5 was fixed as significant.

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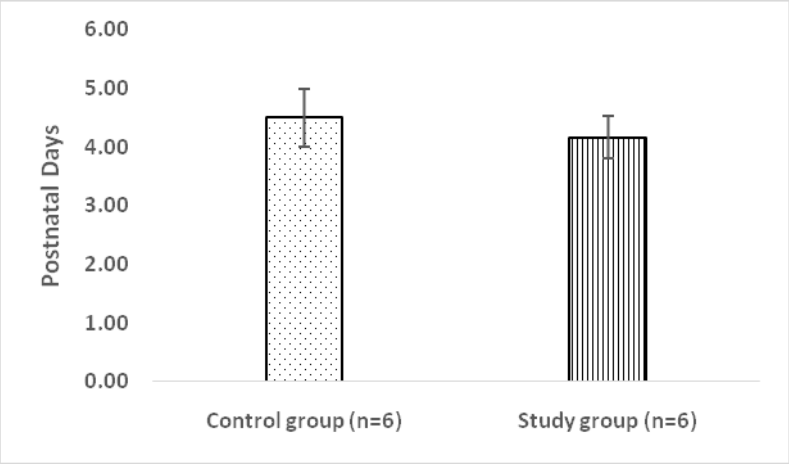
Graph **2:** Comparison of ear opening time between control and study groups



Graph **2:** Comparison of ear opening time between control and study groups

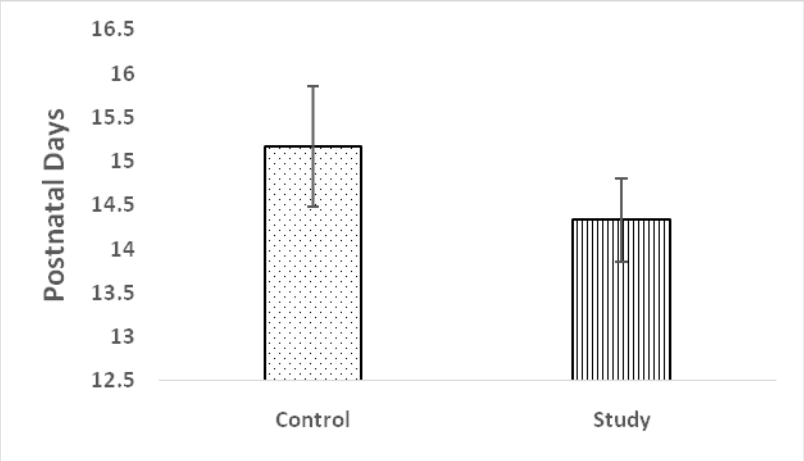


Table 3: Geotaxis test

Score (0,1,2)	Control group (n=6)	Study group (n=6)	P value
7th PND	1.17±0.69	1.67±0.47	0.10
9th PND	2.00±0.00	2.00±0.00	0.00

P<0.5 was fixed as significant.

Graph 3: **Geotaxis test**

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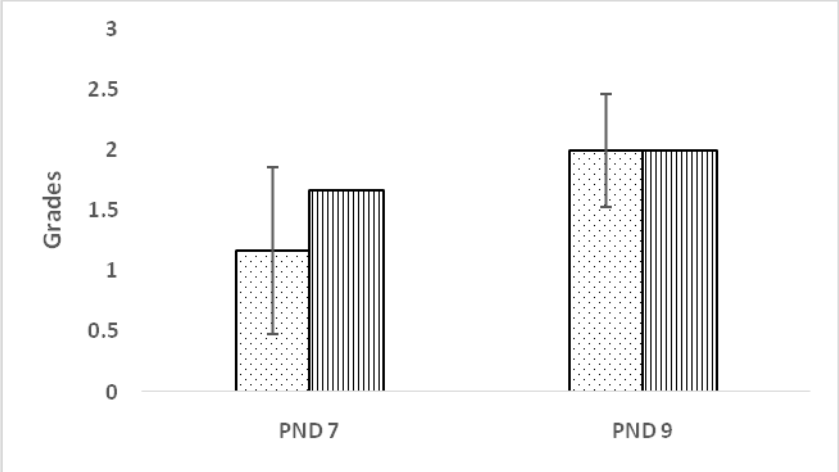
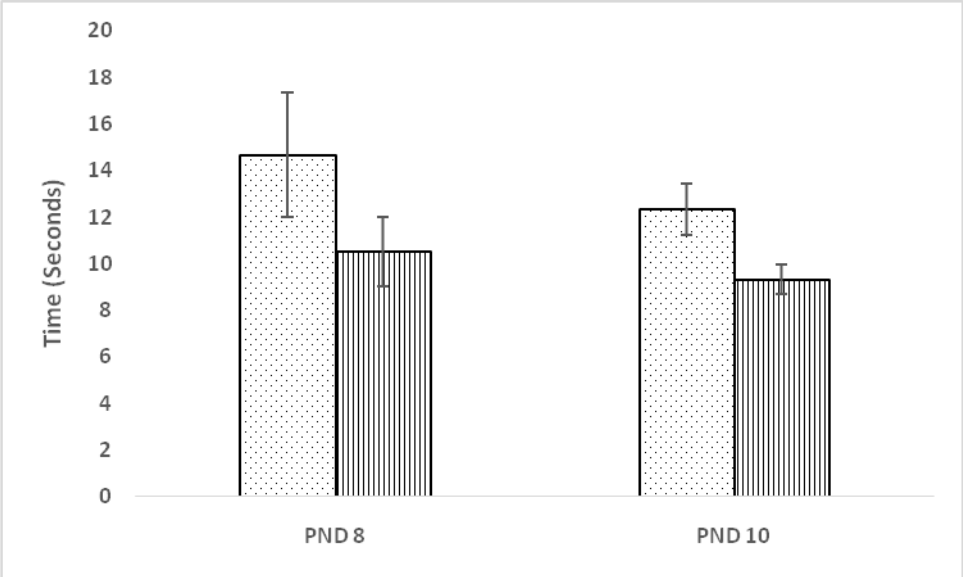


Table 4: Olfactory discrimination test

Latency time	Control group (n=6) (seconds)	Study group (n=6) (seconds)	P value
8th PND	14.67±2.69	10.50±1.12	0.005
10th PND	12.33±1.49	9.33±0.65	0.001

P<0.5 was fixed as significant.

Graph 4: **Olfactory discrimination test**



4. Discussion

In this study A.calamus exposed rat off springs got significant weight gain when compared to control group. The physiological mechanism behind increased weight could be due to appetite stimulant action of A.calamus. Previous studies mentioned that Rhizome powder of this plant has been used as a therapeutic agent for anorexia nervosa and it also stimulates metabolism [11].

Average days of eye opening and ear opening were better in study group than control though it was not significant. Geotaxis test which measures the function of vestibular apparatus and cerebellum, mean scores of both groups were similar, there was no significant changes. The olfactory function which was measured by olfactory discrimination test revealed the significant changes in study group. Since there is no study to relate olfactory function with A.calamus, the supportive findings were not there. But better development of olfactory function is considered as a sign of maturation. A.calamus improved Neurological functional score(rota rod performance and grid walking) of middle cerebral artery occlusion (MCAO)–induced ischaemia in rats [12]. These results could be due to presence of phytochemical beta asarone. Previous study confirmed that b -asarone, a component isolated from essential oil of A.calamus, is easy to pass through blood–brain barrier, and brain is an important organ of distributing of it [13]. Recent evidence has revealed the NMDA (N-methyl-D-aspartate) receptor blockade activity of asarone in against N-Methyl-D-aspartic acid (NMDA) or glutamate-induced excitotoxicity [14]. A.calamus has neuroprotective effect against restraint stress-induced neuronal damage. Post stress induction, treatment with A.calamus enhanced the Na+, K+ -ATPase in brain homogenate [15].

Excitatory GABA in the immature brain is thought to play a crucial role in many developmental processes including neuronal differentiation and dendritic arborisation [14, 15]. Na^+ channel blockade and activation of $GABA_A$ receptors provide a possible mechanism for the known anti-epileptic effects of α-asarone [16]. Neurobehavioral changes produced by acryl amide were prevented with the treatment by A.calamus rhizome extract [17]. The action of A.calamus such as inflence over GABA, NMDA recepter and Na+, K+ -ATPase in brain may be helpful for the development of CNS in rat offsprings.

5. Conclusion

Ethanolic extract of A.calamus improves appetite in young rat offsprings. It also promotes olfactory function. But caution should be noted when extrapolating studies performed in the immature rat. In future elaborate studies are needed to explore the mechanism of action of A.calamus in early days of young rats.

Acknowledgements

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References

1. Rajput SB, Tonge MB, Karuppayil SM. An overview on traditional uses and pharmacological profile of Acorus calamus Linn. (sweet flag) and other Acorus species. Phytomedicine. 2014;21(3):268-276.
2. Mythili Avadhani MN et al. The Sweetness and Bitterness of Sweet Flag [Acorus calamus L.] – A Review. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2013;4(2):598-610.
3. Hao ZY, Liang D, Luo H, et al. Bioactive sesquiterpenoids from the rhizomes of Acorus calamus. J Nat Prod. 2012;75(6):1083-1089.

4. Hazra R, Ray K, Guha D. Inhibitory role of Acorus calamus in ferric chloride-induced epileptogenesis in rat. Hum Exp Toxicol. 2007;26(12):947-953.18375638
5. Yutao GENG, et al. Beta-Asarone Improves Cognitive Function by Suppressing Neuronal Apoptosis in the Beta-Amyloid Hippocampus Injection Rats. Biol.pharm.Bull,2010;33(5):836-845.
6. Karta Purkh Singh Khalsa, Herbal Medicines Benefit Autism, Journal of the American Herbalists Guild. 2015: 13(2),1-9
7. Shah PD, Ghag M, Deshmukh PB, Kulkarni Y, Joshi SV, Vyas BA, et al. Toxicity study of ethanolic extract of Acorus calamus rhizome. Int J Green Pharm 2012;6:29-35.
8. Esfandiari E, Ghanadian M, Rashidi B, Mokhtarian A, Vatankhah AM. The effects of Acorus calamus L. in preventing memory loss, anxiety, and oxidative stress on lipopolysaccharide-induced neuroinflammation rat models. International Journal of Preventive Medicine. 2018;9.
9. Altman J, Sudarshan K. Postnatal development of locomotion in the laboratory rats. Anim Behav 1975:2(3): 896–920.
10. Gregory EH, Pfaff DW. Development of olfactory-guided behavior in infant rats. Physiol Behav 1971:6: 573–576.
11. Ranjan et al. Acorus Calamus L.: An Insight Review of Botany, Chemistry, Medicinal Uses and Cultural Practice. J. Chem. Bio. Phy. Sci. 2016:6(3); 1027-1045
12. Shukla PK, Khanna VK, Ali MM, Maurya R, Khan MY, Srimal RC. Neuroprotective effect of Acorus calamus against middle cerebral artery occlusion–induced ischaemia in rat. Human & experimental toxicology. 2006 Apr;25(4):187-94
13. Cho J, Kim YH, Kong JY, Yang CH, Park CG: Protection of cultured rat cortical neurons from excitotoxicity by asarone, a major essential oil component in the rhizomes of Acorus gramineus. Life Sci 2002;71(5): 591-599
14. Reddy S. et al: Acorus Calamus Rhizome Extract on Neuromodulatory System in Restraint Stress Male Rats. Turk Neurosurg 2015; 25(3):425-431
15. Ben-Ari Y, Khalilov I, Kahle KT, Cherubini E. The GABA excitatory/inhibitory shift in brain maturation and neurological disorders. The Neuroscientist. 2012; 18:467–486.
16. Tyzio R, Holmes GL, Ben-Ari Y, Khazipov R. Timing of the developmental switch in GABAA mediated signaling from excitation to inhibition in CA3 rat hippocampus using gramicidin perforated patch and extracellular recordings. Epilepsia. 2007; 48:96–105.
17. Ze-Jun Wang, Simon R. Levinson,Thomas Heinbockel et al., Identification of both $GABA_A$ receptors and voltage-activated Na^+ channels as molecular targets of anticonvulsant α-asarone.font Pharmacol.2014;5:40
18. Shukla PK, Khanna VK, Ali MM, Maurya RR, Handa SS, Srimal RC. Protective effect of acorus calamus against acrylamide induced neurotoxicity.Phytother Res. 2002 May;16(3):256-60.
19. Differential expression of Helios, Neuropilin-1 and FoxP3 in head and neck squamous cell carcinoma (HNSCC) patients A.A.Mohamed Adil, Anil Kumar Bommanabonia, Anandraj Vaithy, Sateesh Kumar 3biotech 9 (178)
20. Protagonist of Immuno-Profiling, Immuno-Scoring, and Immunotherapy Towards Colitis-Associated Cancer: Systematic Review, Mohamed Adil a.a, AK Pandurangan, M

Waseem, N Ahmed Diagnostic and Treatment Methods for Ulcerative Colitis and Colitis 2020

21. Emerging Role of Mitophagy in Inflammatory Diseases: Cellular and Molecular Episodes, Mohamed Adil AA, S Ameenudeen, A Kumar, S Hemalatha, N Ahmed, N Ali 2020 Curr Pharm Des. 2020;26(4):485-491. doi: 10.2174/1381612826666200107144810
22. Increased Expression of TGF-β and IFN-γ in Peripheral Blood Mononuclear Cells (PBMCs) Cultured in Conditioned Medium (CM) of K562 Cell Culture AAM Adil, L Vallinayagam, K Chitra, S Jamal, AK Pandurangan, N Ahmed Journal of Environmental Pathology, Toxicology and Oncology 38 (2)
23. Cancer immunotherapy: Targeting immunosuppressive tumor microenvironment NA A.A Mohamed Adil Oncobiology and Targets 2014