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Adaptogenic (antistress) Activity of bioactive fractions of *Acorus calamus* (Vacha)

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ABSTRACT

Dried and powder rhizome of *Acorus calamus* (Araceae) was used to isolate the chemical constituent using phase column chromatography and TLC purification. Structure was elucidated by spectroscopic methods. For the evaluation of antistress activity, groups of rats (n = 6) were subjected to forced swim stress one hour after daily treatment of *A. calmus* extract. Urinary vanillylmandelic acid (VMA) and ascorbic acid were selected as non-invasive biomarkers to assess the antistress activity. The 24 h urinary excretion of vanillylmandelic acid (VMA) and ascorbic acid were determined by spectrophotometric methods in all groups under normal and stressed conditions. No change in the urinary excretion of VMA and ascorbic acid was observed in normal animals at all the doses studied.

Keywords: Adaptogenic activity, Stress, *Acorus calamus*, Vanillylmandelic acid, Ascorbic acid

INTRODUCTION

Stress basically is a reaction of mind and body against change in the homeostasis. [1] Stress is a biological response to aversive conditions such as injury, emotional disturbances. [2] This response consists of reactions that tend to threaten or perturbs the homeostasis of the organisms. Stress represents a reaction of the body to a stimulus that tends to alter its normal physiological equilibrium or homeostasis and has been defined as a nonspecific response of the body to any demand imposed on it. [3] The prevalence of cognitive impairment increases expo-nentially with advancing age, and the numbers of affected individuals are increasing due to demographic and other trends. Anxiety frequently presents as a co- morbidity, and as the anxiolytic drugs currently available generally have sedative properties, this poses problems of clinical management. [4] Since the introduction of adaptogens, several plants have been investigated, which were once used as tonics due to their adaptogenic and rejuvenating properties in traditional medicine. The drugs of plant origin are gaining increasing popularity and are being investigated for remedies of a number of disorders including antistress (adaptogenic) activity. According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs. [5] The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive

compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. [6] Natural source from the plant extracts are one of the most attractive sources for the treatment of depression. *Acorus calamus* (also called Sweet flag or Calamus , among many common names (Hindi- bach) is a tall perennial wetland monocot of the Acoraceae family, in the genus Acorus is a famous plant for the treatment of the disease. [7] The roots and rhizomes of *Acorus calamus* (AC) have been used in the treatment of various ailments in ancient Indian, Chinese and other Asian systems of medicine for hundreds of years. It is commonly distributed throughout Europe, temperate India and the Himalayan region. Phytochemically, AC contains monoterpenes, sesquiterpenes, phenlypropanoids, flavonoids, quinine and volatile compounds including αand β-asarone. Aqua-methanolic extract of *Acorus calamus* and its constituent α-asarone has been reported to possess anti-stress activity against noise stress. On the basis of this premise, present study has been designed to evaluate the Antistress and nootropic activities of bioactive fractions of *Acorus calamus* [8]

MATERIALS AND METHODS

Collection and Authentication of Plant material

The rhizomes of *Acorus calamus* was collected from the High Altitude Plant Physiology Research Centre (HAPPRC), Srinagar, Uttarakhand, India in the month of July 2016 and deposited in National Botanical Research Institute, Lucknow, India for taxonomical authentication. The rhizomes were air dried for 20 days and crushed into coarse powder with a grinder and passed through 40-mesh sieve. They were stored in a well closed container separately.

Animals

Swiss albino mice (20-25 g) were bought from the Animal House of A- One Pharmacy College, Ahmedabad, India. The animal room was maintained on a 12-h light and dark cycle with a constant temperature and humidity. Standard pellet food and tap water were available adlibitum. All animal studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the Department of Pharmacology of the A- One Pharmacy College, Ahmedabad, India. The experiments were conducted in a sound proof laboratory. All the experimental procedures and protocols (IAEC / CPCSEA Proposal number 03) used in the study were reviewed by the Institutional Animal Ethics Committee.

Extraction and isolation of compound from rhizomes of *Acorus calamus*

The air dried and powder rhizome (2 kg) of *Acorus calamus* was extracted with ethanol: water (60:40, v/v) under reflux at room temperature. After exhaustive extraction the combined extracts were concentrated under reduce pressure. A known quantity of the crude hydroalcoholic extract (919 g) was dispersed in distilled water (100ml). Further, this dispersion was transferred to separating funnel and extracted three times with 20 ml of n-hexane. The mixture was shaken vigorously for 5 minutes, allowing the air to escape out. It was kept for about 30 min to separate out two layers. The upper layer of nhexane was collected. Each fraction was evaporated to dryness under reduced pressure. The yield of n-hexane extract was found to be 296.2 g. The remaining aqueous layer was discarded. The n-hexane fraction was subjected to silica gel column 60-G (500 g Merck) [20] and successively eluted with a stepwise gradient of n-hexane- EtOAc (100/0, 95/5, 90/10, 75/25, 50/50 and 0/100, v/v). Column fractions were analyzed by TLC on silica gel 60-G (0.2 mm thick) and fractions with a similar TLC pattern were pooled and concentrated. The active 90/10 elutes which shows three compounds in TLC (Rf: 0.56, 0.63, 0.73) was successively rechromatographed on a silica gel column with using solvents (Toluene: ethyl acetate: chloroform / 6:2:2, v/v/v). Finally obtained fractions was (18-20) having single compound confirmed by TLC (n-hexane: toluene: ethyl acetate / 1:1:0.1, v/v/v). [9]

Antistress activity

Rats of either sex weighing between 120–150 gm were divided into four groups (I, II, III, IV) each containing six animals. The 24 h urine sample from each group was collected into two different beakers, one containing 5 ml of 10% oxalic acid for the spectrophotometric determination of ascorbic acid at 550 nm and the other containing 0.5 ml of 6 *N* hydrochloric acid for the determination of vanillylmandellic acid (VMA) spectrophotometrically at 360 nm. The experimental protocol was divided into four phases. In the first phase of the experiment, 24 h urine samples were

collected in all the four groups and subjected to analysis for both VMA and ascorbic acid and the normal values were recorded for seven consecutive days. In the second phase, the animals in each group were subjected to fresh water swimming stress individually. In this method, rats were forced to swim until exhausted (three to four minutes) in a cylindrical vessel of 60 cm height and 45 cm diameter containing water at room temperature (28°C). Water depth was always maintained at 40 cm. The 24 h urinary levels of VMA and ascorbic acid under stressed conditions were determined again as described above daily for seven consecutive days. The third phase of the experiment consists of administration of *A. calamus* extract to the same groups of animals after having recovered completely to normal condition. Groups II, III and IV were administered orally with *A. calamus* (suspended in 2% gum acacia) at daily doses of 100, 200 and 300 mg/kg body weight respectively for seven consecutive days while group I serving as control. The 24 h urine samples were collected and the levels of both VMA and ascorbic acid were determined. The final phase of the experiment consisted of administration of *A. calamus* extract to the same groups of animals after a recovery period of one week. Groups II, III and IV were administered orally with *A. calamus* at doses of 100, 200 and 300 mg/kg body weight respectively, one hour prior to the daily induction of stress for seven consecutive days while group I serving as control. The 24 h urine samples were collected and analyzed for VMA and ascorbic acid for seven consecutive days to study the influence of the extract on the stress induced biochemical changes. [10]

Statistical analysis

Results are expressed as mean±standard error of mean (SEM). The data for anti-stress activity were subjected to the Student's paired *t* test. The data for AChE inhibitory activity was subjected to one way ANOVA followed by Dunnett's test. In all tests, the criterion for statistical significance was $p<0.05$.

RESULTS AND DISCUSSION

Rats when forced to swim in a restricted space become immobile after a certain period of vigorous activity which indicates a state of mental depression. An increase in total swimming time indicates better stress tolerance. The urinary data of VMA and ascorbic acid observed in various phases of the experiment are shown in Table 1 and Table.2 respectively. Induction of forced swim stress to the animals produced a significant increase in VMA and decrease in ascorbic acid excretion compared to their respective basal excretion in normal condition. Both the parameters were found to return to their normal levels in three to four days after withdrawal of stress. Induction of forced swim stress to the animals brings about a significant increase ($P<0.05$) in VMA level and decrease ($P<0.05$) in ascorbic acid excretion when compared to their basal excretion in normal condition. Both the parameters were regained within four days after withdrawal of stress. Daily treatment of to the animals under normal condition did not produced any change in the excretion of VMA and ascorbic acid compared to normal basal levels which indicates that there was no alteration in the excretion of VMA and ascorbic acid under normal condition. Daily administration of *the extract* one hour prior to the induction of stress inhibited the increase in VMA and decrease in ascorbic acid excretion which was manifested during stress alone. The inhibition of both the parameters i.e.increase in VMA levels and decrease in ascorbic acid levels was significant at all dose levels ($p < 0.05$) in a dose dependent manner. The urinary data of VMA and ascorbic acid observed in various phases of the experiment are shown in Fig. 1 and Fig. 2 respectively. Induction of forced swim stress to the animals produced a significant increase in VMA and decrease in ascorbic acid excretion compared to their respective basal excretion in normal condition. Both the parameters were found to return to their normal levels in three to four days after withdrawal of stress. Daily treatment of *A. calamus* to the animals under normal condition produced no change in the excretion of VMA and ascorbic acid compared to normal basal levels indicating that *A. calamus* did not alter excretion of VMA and ascorbic acid in normal condition. Daily administration of *A. calamus* one hour prior to the induction of stress inhibited the increase in VMA and decrease in ascorbic acid excretion which was manifested during stress alone. The inhibition was found to be significant at all dose levels in a dose dependent manner.

Table 1. Effect of methanolic extract on 24hr urinary levels of VMA in normal and stress-induced rats

Treatment	Group I	Group II	Group III	Group IV
Normal	194.90±0.4881	194.60±0.2226	185.40±0.5714	194.40±0.2965
Stress	379.50±0.2916	380.00±0.4099	289.60±0.5379	291.80±0.7518
Normal+Extract	198.70±0.6039	194.10±0.47	186.40±0.42	194.80±0.25
Extract+Stress	389.10±0.7747	279.80±0.40	194.10±0.47	194.90±0.29

Table 2.Effect of methanolic extract on the 24hr urinary level of Ascorbic acid in normal and stress-induced Rats

Treatment	Group I	Group II	Group III	Group IV
Normal	146.80±0.5483	152.20±0.8634	138.50±0.53	145.00±0.4641
Stress	73.65±0.5204	79.94±0.4099	61.82±0.6267	61.94±0.4864
Normal+Extract	143.00±0.5447	154.00±0.5158	143.60±0.5695	144.70±0.249
Extract+Stress	76.78±0.69	146.20±0.3581	133.00±0.6851	148.10±0.3959

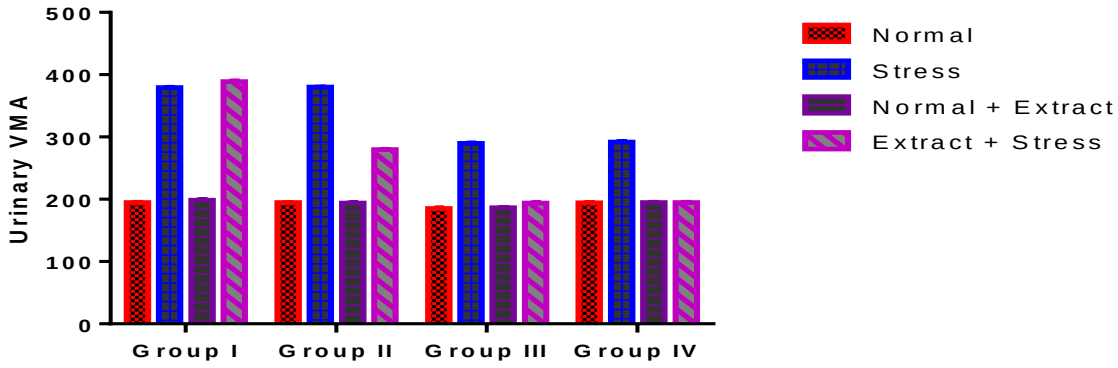

Fig 1.Effect of methanolic extract on 24hr urinary levels of VMA in normal and stress-induced rats

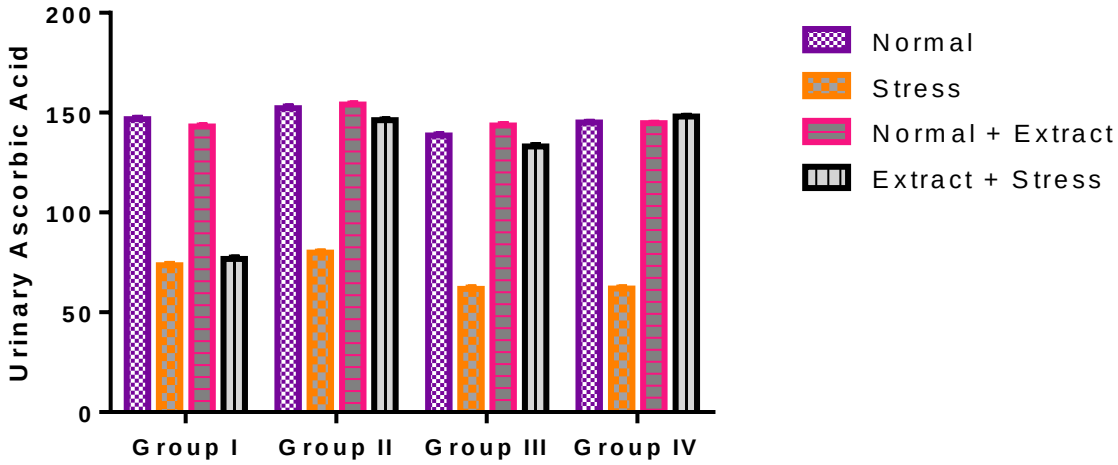

Fig 2. Effect of methanolic extract on the 24hr urinary level of Ascorbic acid in normal and stress-induced rats

CONCLUSION

This study provided evidence for antioxidant, anti-stress of the selected bioactive extract and that use of them by humans as nutraceuticals is beneficial and scientific. In conclusion, the present study provides scientific support for the antistress (adaptogenic), *Acorus calamus* extract and substantiate the traditional claims for the usage of grape fruits and seeds in stress induced disorders. Further investigations are required to characterize *Acorus calamus* the active constituent(s) responsible for observed activities of the extract.

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