

EXAMINATION OF CNS ACTIVITY OF RHIZOMES OF *ALPINIA OXYPHYLLA*Vimal Singh Chauhan^{1*} and Swapna¹¹Department of Pharmacology, Vidhya Bharathi College of Pharmacy, BITU, Rourkela, Odisha, India, 769015.Corresponding author: E-mail: cvimalsingh@gmail.com

ABSTRACT: The study is based on the examination of the CNS activity observed from the methanolic extract of the rhizomes of *Alpinia oxyphylla*. Tail immersion method in mice has been used for the evaluation of the central pharmacological actions. Similarly acetic-acid induced writhing-test was used for the evaluation of the peripheral pharmacological properties. A significant rise in pain threshold is seen in a dose dependent manner with the methanolic extract of *A. oxyphylla* at doses of 100, 200 and 400 mg/kg body weight with the tail immersion methods. The methanolic extract at 400 mg/kg dose possessed 73.12% writhing inhibition, ($p < 0.001$) in acetic-acid induced writhing-test that could be compared to the standard, Diclofenac-Na (25 mg/kg) with 75.78% inhibition. Open-field and hole-cross tests have been conducted in mice for further investigation of the extract in support of its neuro-pharmacological actions, where dose-dependent suppression of exploratory and motor activities were observed in the tested models. Hence, the above results evidence the presence of CNS depressant and analgesic properties of the plant, *A. oxyphylla*.

Key words: *Alpinia oxyphylla*, tail-immersion test, methanolic extract, acetic-acid induced writhing-test.

INTRODUCTION

Plants of the genus *Alpinia* Zingiberaceae family are used as traditional herbs (Kong *et al.*, 2000) in some areas of the peoples of republic of china and India for relieving stomach ache (Tang *et al.*, 1992), treating colds, invigorating the circulatory system, and reducing swellings. Previously in *Alpinia* genus diterpenoids, flavonoids, oxygenated sesquiterpenoids and phenyl propanoids were reported. The rhizomes of *Alpinia oxyphylla*, which have a strong aromatic odour, is a well-known crude drug used as an aromatic stomachic in China and Japan (Itokawa *et al.*, 1980). Essential oils, extracts and their constituents of plants are greatly valued in ayurveda and have been also reported to exhibit a wide range of biological activities of therapeutic importance that include antiseptic activity, antimicrobial (Janseen *et al.*, 1985), osteoarthritis of the knee (Altman *et al.*, 2001), antitumor, antiulcer (Matsuda *et al.*, 2003), diabetes, anthelmintic activity (Raj *et al.*, 1975), certain heart problems, central nervous system disorders (White *et al.*, 2014, Fajemiroye *et al.*, 2014, Polepally *et al.*, 2013, Prabhakar *et al.*, 2014, Zjawiony *et al.*, 2011), gastrointestinal disorders (Salaga *et al.*, 2014), cough and bronchitis anti HIV (Raju *et al.*, 2008), and anticancer. It is widely cultivated in India and South East Asian countries. Though the plant is traditionally used in many parts of India, no scientific report is available to validate the folkloric use. Again, plants have been a promising source of drug molecules for ages. Still the untapped wealth of plant kingdom is a major target for the search of new lead compounds in drug discovery. In India, huge number of plants still remains unexplored. So well designed, systematic and objective research in this area might benefit our people who have been deluged with superfluity of disease, and who lack technological and economic resources. In present study, we investigated the analgesic and central nervous system depressant activities of methanolic extracts of *A. oxyphylla*.

MATERIALS AND METHODS

Plant material:

The rhizomes of *A. oxyphylla* were collected from the forest of area of Rourkela, Odisha, during the first week of January, 2014, was identified by Dr. Abhijith Chauhan, Taxonomist, Dept. of Taxonomy, BITU, Rourkela, Odisha. Collected seeds, after cutting into small pieces, were dried and pulverized into a coarse powder and stored into an air-tight container.

Extraction and sample preparation

The pulverized coarse powder of seeds of *A. oxyphylla* (500 gm) was extracted with methanol by successive cold extraction. The extracts obtained, were filtered off and evaporated to dryness in an oven at low temperature. The extracts rendered concentrate of reddish color.

Animal

For the experiment both male and female Swiss albino mice, 3-4 weeks of age, weighing between 20-25 gm, were collected from the animal research branch of the National Center of Animals and Research (NCAR), Odisha. Animals were maintained under standard environmental conditions (temperature: $24.0 \pm 1.0^\circ\text{C}$), relative humidity: 55-65% and 12 hrs light/12 hrs dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experimentation.

Analgesic activity

Tail immersion test

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice. The animals were treated as discussed above 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C . The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 12s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined at 0, 30, 60 and 90 min after the administration of drugs. Percentage of elongation was calculated using the following formula.

$$\text{Elongation (\%)} = \frac{\text{Latency (Test)} - \text{Latency (Control)}}{\text{Latency (Test)}} \times 100$$

Acetic Acid-Induced Writhing Test:

The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and Control were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was intra-peritoneally administered 15 min before injection of acetic acid and Diclofenac-Na (25 mg/kg) was used as standard drug. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min (Ahmed *et al.*, 2004). Percentage inhibition of writhing was calculated using the following formula.

$$\text{Writhing inhibition (\%)} = \frac{\text{Mean No. of writhing (control)} - \text{Mean No. of writhing (test)}}{\text{Mean No. of writhing (control)}} \times 100$$

Neuropharmacological activity:

Hole cross test:

A steel partition was fixed in the middle of a cage having a size of $30 \times 20 \times 14$ cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage (Takagi *et al.*, 1971). The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after the oral treatment with *A. oxyphylla* methanolic extracts at the doses of 100, 200 and 400 mg/kg body weight (table 3). Percentage inhibition of movements was calculated using the following formula.

$$\text{Movements inhibition (\%)} = \frac{\text{Mean no. of movements (control)} - \text{Mean no. of movements (test)}}{\text{Mean No. of movements (control)}} \times 100$$

Open field test

The animals were divided into control, standard, and test groups containing five mice each. The test group received *A. oxyphylla* extract at the doses of 100, 200 and 400 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water) and standard group received Diazepam (3 mg/kg body weight). In the open field test, the floor is half square meter that was divided into a series of alternatively colored black and white squares. The apparatus also include a wall of 40 cm height. The number of squares visited by the animals will be counted for 3 min at 0, 30, 60, 90, and 120 min after oral administration of the test drugs (Gupta *et al.*, 1971). Percentage inhibition of movements was calculated using the following formula.

$$\text{Movements inhibition (\%)} = \frac{\text{Mean no. of movements (control)} - \text{Mean no. of movements (test)}}{\text{Mean No. of movements (control)}} \times 100$$

Statistical Analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple-comparisons. The obtained results were comparable with the vehicle control group. *p* values < 0.05, 0.001 were considered to be significant statistically.

RESULTS

The analgesic activity of the methanolic extract of *A. oxyphylla* was evaluated in tail immersion and acetic acid-induced writhing methods. The tail withdrawal reflex time following administration of the extract of *A. oxyphylla* was found to increase with increasing dose of the sample. In this test, the maximum effect was seen after the administration of the drug at 60 and 90 min. The result was significant statistically (*p*<0.05-0.001) and was comparable to the control. The result was statistically significant (*p*<0.05-0.001) and was comparable to the control (Table 1).

Table 1: Effects of the Methanolic Extract of *A. oxyphylla* on tail withdrawal reflex of mice induced by Tail immersion method

Groups	Dose (mg/kg)	Mean reaction time(s) before and after drug administration (% of tail flick elongation)			
		0 min	30 min	60 min	90 min
Control		1.73 ±0.125	1.60±0.125(-)	1.47±0.17 (-)	1.33±0.105 (-)
Standard	25	2.53±0.29	5.33±0.235** (69.98%)	7.39±0.07 ** (80.10%)	8.8±0.17**(84.88%)
Group – I	100	1.82±0.02	4.45±0.385 (64.04%)	6.09± 0.405**(75.86%)	7.06±0.50** (81.16%)
Group – II	200	1.86±0.035	5.53±0.335** (71.06%)	6.28 ±0.495** (76.59%)	7.44±0.305** (82.12%)
Group – III	400	1.82±0.05	6.50±0.24** (75.68%)	7.38±0.325** (80.08%)	9.88±0.495** (86.53%)

Control: animals received (1% Tween 80 in water), Standard group received Diclofenac-Na (25 mg/kg body weight i.p.), Group-I, Group-II and Group – III were treated with 100, 200 and 400 mg/kg body-weight of the extract per oral. Values are mean ±SEM, (n=5); ***p* <0.001, Dunnett's test as compared to control

The doses of the extract significantly (*p* < 0.001) inhibited writhing response induced by acetic acid in a dose dependent manner as compared to control. At 100 mg/kg body weight the extract showed 26.15% inhibition, at 200 mg/kg body weight the extract showed 47.94% inhibition and at 400 mg/kg body weight showed 73.12% inhibition of writhing compared to the standard drug Diclofenac-Na which showed 75.78% inhibition of writhing at 25 mg/kg body weight dose (Table 2).

The extract at doses level of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight showed significant (*p* < 0.001) decrease of movement from its initial value during the period of hole cross experiment as compared to control (Table 3). The maximum decrease in movement was observed at 90 and 120 min after drug administration. In the open field test at dose level of 100mg/kg, 200mg/kg and 400mg/kg body weight the number of squares traveled by the mice was suppressed significantly from its initial score by both doses of the extract which is comparable to the standard drug Diazepam (Table 4). The maximum suppression was exhibited at 90 and 120 min after drug administration.

Table 2: Effect of *A. oxyphylla* Methanolic Extract on Acetic Acid induced writhing in Mice

Group	Treatment and Dose	Writhing (Mean \pm SEM)	% of writhing	% of writhing inhibition
Control	0.7% acetic acid (10ml/ kg, i.p.)	41.3 \pm 1.32	100.00	0
Standard	Diclofenac sodium (25 mg/kg i.p.)	10.0 \pm 0.42**	24.21	75.78
Group – I	Extract (100 mg/kg per oral)	30.5 \pm 1.035**	73.85	26.15
Group – II	Extract (200 mg/kg per oral)	21.5 \pm 0.995**	52.06	47.94
Group – III	Extract (400 mg/kg per oral)	11.1 \pm 2.88**	26.87	73.12
Diclofenac sodium was administered 15 min before 0.7% acetic acid administration. Writhing was counted for 15 min, starting after 5 min of acetic acid administration; **p<0.001 vs. control, values are mean \pm SEM; (n=5)				

Table 3: Effect of *A. oxyphylla* Methanol Extract on Hole Cross Test in Mice

Group	Dose (mg/ kg)	Number of movements (% of Number of movements inhibition)				
		0 min	30 min	60 min	90 min	120 min
Control		22.4 \pm 1.63	11.8 \pm 0.66 (-)	11.4 \pm 0.74 (-)	7.8 \pm 0.86 (-)	10.2 \pm 0.37 (-)
Standard	3	15.2 \pm 1.11	6.6 \pm 1.66* (44.07%)	4.0 \pm 1.09** (64.91%)	2.4 \pm 1.25* (69.23%)	1.6 \pm 0.87** (84.31%)
Group – I	100	14 \pm 2.74	9.4 \pm 1.63 (20.3%)	7.6 \pm 2.065 (33.33%)	4.6 \pm 1.53 (41.03%)	1.4 \pm 0.51* (86.24%)
Group – II	200	15 \pm 0.83	6.6 \pm 0.60*(44.07%)	5.2 \pm 0.20* (54.38%)	3.2 \pm 0.91* (58.97%)	2.0 \pm 0.54** (80.39%)
Group –III	400	14.2 \pm 4.3	7.2 \pm 2.22 (38.98%)	2.4 \pm 0.98** (78.95%)	1.4 \pm 0.74** (82.02%)	0.6 \pm 0.24** (94.12%)
Control: animals received (1% Tween 80in water), Standard group received Diazepam 3 mg/Kg body weight i.p., Group-I, Group-II and Group – III were treated with 100, 200 and 400 mg/kg body weight of the crude extract of <i>A. oxyphylla</i> per oral. Values are mean \pm SEM, (n=5); *p <0.05, **p<0.001, Dunnett's test as compared to control						

Table 4: Effect of *A. oxyphylla* Methanolic Extract on Open Field Test in Mice

Group	Dose (mg/kg)	Number of movements (% of number of movements inhibition)			
		0 min	30 min	60 min	90 min
Control		113 \pm 3.22	106.6 \pm 1.69(-)	91.2 \pm 1.53(-)	87.4 \pm 1.63 (-)
Standard	3	83.2 \pm 14.21	39.4 \pm 8.14** (63.03%)	32.6 \pm 6.22** (64.25%)	24.2 \pm 6.9 ** (72.31%)
Group – I	100	52 \pm 6.26	30.2 \pm 3.12** (71.67%)	25.2 \pm 2.22** (72.37%)	22.6 \pm 2.27** (74.14%)
Group – II	200	66.6 \pm 5.78	45.2 \pm 9.78** (57.60%)	27 \pm 7.98** (70.39%)	4.4 \pm 2.99** (94.96%)
Group – III	400	51.4 \pm 14.46	30.6 \pm 3.4** (71.29%)	19.2 \pm 0.735** (78.94)	10.2 \pm 1.67** (88.32%)
Control: animals received (1% Tween 80in water), Standard group received Diclofenac –Na (25 mg/Kg body weight i.p.), Group-I, Group-II and Group – III were treated with 100, 200 and 400 mg/kg body weight of extract per oral. Values are mean \pm SEM, (n=5); **p <0.001, Dunnett's test as compared to control					

DISCUSSION

Acetic acid induced writhing test is suitable for detecting both central and peripheral analgesia, whereas tail flick tests are most sensitive to centrally acting analgesics. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic mediators like PGE₂ and PGF₂ α and their levels increase in the peritoneal fluid of the acetic acid induced mice. The abdominal constrictions produced after administration of acetic acid is related to sensitization of nociceptive receptors to prostaglandins. It is therefore possible that the extract exerts its analgesic effect by inhibiting the synthesis or action of prostaglandins which may be due to phytochemicals present in the extract. Thermally induced nociception indicates narcotic involvement. The centrally acting analgesics generally elevate the pain threshold of mice towards heat. The extract significantly delayed the response time to thermal pain sensation in tail flick method indicating narcotic involvements. Moreover, since the extract inhibited both peripheral and central mechanisms of pain, it is possible that the extract acted on opioid receptor (Elisabetsky *et al.*, 1995, White *et al.*, 2014, Fajemiroye *et al.*, 2014, Polepally *et al.*, 2013, Prabhakar *et al.*, 2014, Zjawiony *et al.*, 2011). While evaluating neuro-pharmacological activities of *A. oxyphylla*, it was found that the plant extract possesses central nervous system depressant activity as indicated by decreased exploratory behavior in mice Fujimori *et al.*, 1995). Results of the present investigation suggest that the extract of *Alpinia oxyphylla* possesses strong analgesic and CNS depressant activity and provide a scientific basis for the use of the plant in traditional system of medicine in the treatment of inflammatory disorders.

CONCLUSION

From the above results, it could be concluded that the plant extract of *A. oxyphylla* is known to possess significant CNS depressant and analgesic properties that could be mediated from the depression in the central mechanism of pain, thereby lending support for the use of the plant in inflammatory and pain-disorders. However, there is need for further studies in understanding the exact mechanisms underlying in the novel compound(s) yet to be isolated that could be responsible for the CNS depressant and analgesic properties.

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