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STUDY OF THE ANTIDEPRESSANT AND ANTINOCICEPTIVE ACTIVITY OF ETHANOLIC EXTRACT OF RHIZOMES OF *ZINGIBER OFFICINALE* IN EXPERIMENTAL ANIMALS

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Keywords:

Antidepressant property, antinociceptive activity, *Zingiber officinale*, forced swim test, writhing

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ABSTRACT: Objective: Ginger (*Zingiber officinale* Roscoe), a well-known spice plant, has been used traditionally in the treatment of a wide variety of ailments. The main objective of the work is to evaluate the antidepressant and antinociceptive activity of ethanolic extract of *Zingiber officinale* in Swiss albino mice. **Methods:** The present study was design to evaluate the antidepressant property of *Zingiber officinale* as well as its interaction with conventional antidepressant drug using forced swim test and to evaluate possible mechanism involved in its action. Antinociceptive activity was evaluated by acetic acid induced writhing and its interaction with conventional analgesic drug was also noted. The rhizomes of ginger were authenticated and extraction of dried rhizomes was done by using soxhlet apparatus to obtain its ethanolic extract. **Results:** The alcoholic extract of *Zingiber officinale* (300mg/kg p.o and 150mg/kg p.o) for antidepressant activity significantly reduced the immobility time in mice (P<0.05) as compared to the vehicle control. The extract of *Zingiber officinale* (100 and 200 mg/kg, p.o.) significantly suppressed the acetic acid-induced writhing response in a dose-dependent manner in mice (P<0.05) as compared to the vehicle control. **Conclusion:** The plant extract of *Zingiber officinale* showed significant antidepressant activity in forced swim test model and significant antinociceptive effect in acetic acid induced writhing.

INTRODUCTION: Ginger, the rhizome of *Zingiber officinale*, is one of the commonly used species of the ginger family *Zingiberaceae* and is used for various foods and beverages.¹ Although probably a native of Asia, *Zingiber officinale* has become naturalized in many countries and is now widely distributed throughout tropical and subtropical parts of the world. Ginger has been cultivated for thousands of years for medicinal purposes and as a spice.

It is used extensively in traditional medicine to treat cold, fever, headache, nausea, and digestive problems and is also used in western herbal medical practices for the treatment of arthritis, rheumatic disorders, and muscular discomfort.²

The main constituents of ginger are the gingerols, shogaols, paradols, and zingerone.³ The major gingerol and shogaol components present in the rhizome of ginger are 6-gingerol and 6-shogaol, respectively. The main aromadefining component is zingiberol, whereas others such as gingediol, monoacyldigalactosyl-glycerol, diarylheptanoids, and phytosterols have also been identified.⁴ Depression is an important global public health issue affecting mood, thought, behaviour of an individual and is associated with chronic disability. About 21% of world's population is affected.

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Synthetic antidepressants are associated with side effects like dry mouth, constipation etc. but natural medicinal plants may be important sources of novel antidepressant drugs which are devoid of many unwanted side effects. A mechanism involving central nervous system cannot be ruled out as many constituents of ginger antagonize serotonin type-3 receptors, however this has not been clearly documented.⁵

Opioids have long been used for treating moderate to severe pain, but treatment with these drugs leads to side effects such as analgesic tolerance, physical dependence, emesis, constipation, and drowsiness. The management of pain remains a major clinical problem. Therefore, it is essential to find new strategies for enhancing the analgesic effects of opioids and natural herbs that have analgesic properties without hazardous side effects. Activation of the opioid receptor inhibits adenylyl cyclase activity via inhibitory G-proteins, closing voltage-activated calcium channels and reducing the Ca influx, thus inhibiting neurotransmitter release and attenuating pain sensation.⁶ It has been documented that ginger has Ca channel blocking activity.⁷⁻⁹

In addition, ginger and its constituents have some effects on pain-modulating systems.¹⁰ The oil of ginger is a mixture of constituents, consisting of monoterpenes (phellandrene, camphene, cineole, citral, and borneol) and sesquiterpenes (zingiberene, zingiberol, zingiberenol, β -bisabolene, sesquiphellandrene, and others). Few works have reported the properties of ginger essential oil (GEO). However, several types of terpene compounds are known to present antinociceptive activities.¹¹

So the present study was done to evaluate different pharmacological actions of *Zingiber officinale* including antidepressant and antinociceptive activity in Swiss albino mice due to involvement of different active ingredients so as to minimize the side effects of synthetic drugs and to promote the use of a novel natural medicinal plant.

MATERIALS AND METHODS: The present study was conducted in the Department of Pharmacology, Gauhati Medical College and Hospital.

1. Ethical Review: The protocol was submitted to the Animal Ethics Committee, GMCH and the study was conducted after obtaining permission from the Institutional Animal Ethics Committee (Approval no.- MCI 05/2015/48)

2. Experimental animals:

Inclusion Criteria:

Adult Male Swiss albino mice weighing 25-30 gm was used in the study. The animals were fed on commercial rodent pellets and water *ad libitum* during the study. The animals were housed in plastic cages at a controlled temperature of 24 ± 1 °C and 12 hours light and dark cycle. Before conducting the experiment all animals were acclimatized to laboratory condition for 7 days. All experiments were done after following the guidelines on ethical standards for investigation of experimental pain in animals and the guideline for the investigation of experimental seizures in conscious animals.

Exclusion criteria:

Female Swiss albino mice
Neonate albino mice
Albino mice with signs of disease

3. Collection of plant and preparation of extract:

The rhizomes of *Zingiber officinale* was collected from the local market and authenticated by Professor and Head, Department of Botany, Gauhati University. The *Zingiber officinale* rhizomes (ginger) were cut into smaller pieces, dried under shade for 10 days and pulverized to coarse powder using a manual blender. The ginger powder was extracted with ethanol by continuous extraction in a soxhlet apparatus. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains was discarded. The extract was dried under vacuum, stored at room temperature and protected from direct sunlight in the Department of Pharmacology, Gauhati medical College and Hospital.

4. Acute Toxicity Study: Acute toxicity study was carried out according to OECD 425 guidelines.

Reagent: 0.9% normal saline (0.9% NaCl solution)

Indomethacin
Acetic acid
Fluoxetine

Experimental Design:

To demonstrate antidepressant Effect:

After acclimatization animals were randomly divided into 4 groups of six mice each (n = 6).

Group I: Normal saline 25 ml/kg (control),

Group II: Fluoxetine 20mg/kg i.p (standard),

Group III: 150 mg/kg p.o of *Zingiber officinale* rhizome extract.¹²

Group IV: 300 mg/kg p.o of *Zingiber officinale* rhizome extract.¹²

The test samples were given 45 mins prior to Forced Swim Test. Duration of immobility time were taken as a measure of efficacy and compared with the control and Fluoxetine group. All precautions were taken to minimize animals suffering.

Forced Swim Test: The mouse was individually forced to swim inside a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing 15 cm of water maintained at 25 °C). Mice placed in the cylinders for the first time were initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 6 min in the water mice are removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. The duration of immobility was recorded during the last 4 mins. The mouse was considered immobile when it floated motionlessly. The water was changed after each test.¹³

To demonstrate antinociceptive effect:

After acclimatization, animals were randomly divided into 4 groups of six mice each (n = 6).

Group I: Normal saline 25 ml/kg (control),

Group II: Indomethacin 5 mg/kg i.p (standard)¹¹

Group III: 100 mg/kg p.o of *Zingiber officinale* rhizome extract.¹¹

Group IV: 200 mg/kg p.o of *Zingiber officinale* rhizome extract.¹¹

The test samples were given 30 mins prior to acetic acid solution (10 ml/kg, 0.6% i.p.) induced writhing. After 5 mins of acetic acid solution injection, abdominal muscles constriction together with stretching of the hind limbs was counted over a period of 10 min. No. of writhing were taken as a measure of efficacy and compared with the control and indomethacin group. All precautions were taken to minimize animals suffering. Antinociceptive activity was expressed as the percentage of inhibition of writhings compared with control animals.

Antinociceptive activity = {No. of writhings in control - No. of writhings in standard or test drug/ No. of writhings in control}* 100

RESULTS AND OBSERVATION: The data obtained were analyzed and presented using appropriate statistical method. Results were expressed as Mean ± SEM. Data was analyzed by one-way analysis of variance, followed by Dunnett's multiple comparison tests which was performed using primer of biostatistics. $P < 0.05$ was considered as significant.

Antidepressant Property: In the present study, standard anti-depressant fluoxetine at the dose of 20mg/kg significantly reduced the duration of immobility ($P < 0.05$) as compared to the vehicle control group. In forced swim test the extract shortened the immobility period in comparison with control and exhibited a dose dependent antidepressant activity. As shown in the **Table 1**, the alcoholic extract of *Zingiber officinale* at the doses of 300mg/kg and 150mg/kg significantly reduced the immobility time in mice ($P < 0.05$) as compared to the vehicle control.

TABLE 1: EFFECTS OF ZINGIBER OFFICINALE ON DURATION OF IMMOBILITY IN THE FORCED SWIM TEST (FST) USING MICE

Group No.	Group	Dose (mg/kg)	Duration of Immobility (Sec)
I	Control- Normal saline	25 ml/kg	45.67 ± 0.99
II	Standard drug- Fluoxetine	20 mg/kg	18.5 ± 0.42**
III	Test drug- <i>Zingiber officinale</i>	150 mg/kg	32.33 ± 0.8**
IV	Test drug- <i>Zingiber officinale</i>	300 mg/kg	21.16 ± 0.47**

Values represented as Mean ± S.E.M. (n=6), ** $P < 0.05$ vs control (group 1)

The extract at a dose of 300mg/kg body weight is found to be effective nearly similar to that of standard drug fluoxetine. (Fig. 1)

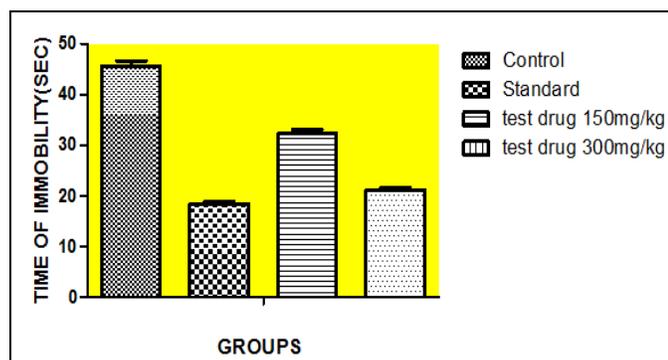


FIG. 2: EFFECTS OF *ZINGIBER OFFICINALE* ON DURATION OF IMMOBILITY IN THE FORCED SWIM TEST (FST) USING MICE

Antinociceptive Property: In the present study, standard drug, indomethacin at the dose of 5mg/kg significantly suppressed the acetic acid-induced writhing response ($P < 0.05$) as compared to the vehicle control group. As shown in the Table 2, the extract of *Zingiber officinale* (100, and 200 mg/kg, p.o.) significantly suppressed the acetic acid-induced writhing response in a dose-dependent manner in mice ($P < 0.05$) as compared to the vehicle control.

TABLE 2: EFFECTS OF *ZINGIBER OFFICINALE* ON NO. OF WRITHING IN ACETIC ACID-INDUCED WRITHINGS RESPONSE USING MICE

Group No.	Group	Dose (mg/kg)	No. of Writhings	Percentage Inhibition (%)
I	Control- Normal saline	25 ml/kg	30.33 ± 1.67	-
II	Standard drug- Indomethacin	5 mg/kg	13.33 ± 0.99**	56.0
III	Test drug- <i>Zingiber officinale</i>	100 mg/kg	23.67 ± 1.49**	21.9
IV	Test drug- <i>Zingiber officinale</i>	200 mg/kg	17.67 ± 1.40**	41.7

Maximum inhibition of *Zingiber officinale* was observed at the dose of 200 mg/kg which is found to be effective nearly similar to indomethacin. Antinociceptive activity was expressed as the percentage of inhibition of writhing compared with control animals which was maximum for indomethacin followed by *Zingiber officinale* extract at a dose of 200 mg/kg. (Fig. 2)

TABLE 2: EFFECTS OF *ZINGIBER OFFICINALE* ON NO. OF WRITHING IN ACETIC ACID-INDUCED WRITHINGS RESPONSE USING MICE

Group No.	Group	Dose (mg/kg)	No. of Writhings	Percentage Inhibition (%)
I	Control- Normal saline	25 ml/kg	30.33 ± 1.67	-
II	Standard drug- Indomethacin	5 mg/kg	13.33 ± 0.99**	56.0
III	Test drug- <i>Zingiber officinale</i>	100 mg/kg	23.67 ± 1.49**	21.9
IV	Test drug- <i>Zingiber officinale</i>	200 mg/kg	17.67 ± 1.40**	41.7

Values represented as Mean ± S.E.M. (n=6), ** $P < 0.05$ vs control (group 1)

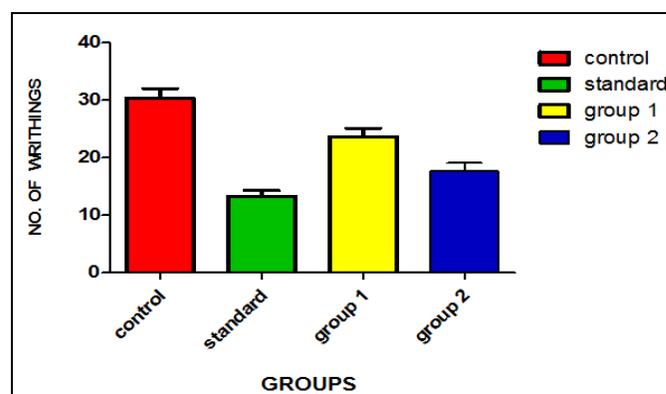


FIG. 2: EFFECTS OF *ZINGIBER OFFICINALE* ON NO. OF WRITHING IN ACETIC ACID-INDUCED WRITHINGS RESPONSE USING MICE

DISCUSSION: Mood disorders are one of the most common mental illness with a lifetime risk of 10% in general population. Around 5% general population is suffered from depression with suicide being one of the most common and dangerous outcome. Most of the drugs which act as antidepressant and are in use now have lots of adverse effects which ultimately lead to non compliance and affect the quality of life. These further complicate the problem. Hence a number of studies are going on drug formulations of plant origin that proved to be effective as well as free of adverse effects of conventional drug therapy.

One of the most important ways to identify new antidepressant drugs are animal models.^{14, 15} The most common method used for the detection of antidepressants in animals is forced swimming test (FST) that was proposed primarily^{16, 17} and was subsequently improved.¹⁸ The FST is the most widely used pharmacological model for assessment of potential antidepressant activity in rodents.

Increased immobility factor in forced swimming test is an indicator for depression like condition in animal. Eventually in test, it was observed that rodents develop immobility when they are placed in cylinder of water after they stop active escape behaviors, such as increased the score of swimming behavior significantly climbing or swimming.

The use of antidepressants in the treatment reduces immobility, or delays its onset, and increase or prolong active escape behaviors displayed during the FST. Experiments have shown that swimming score is sensitive to serotonergic compounds, and that climbing test is sensitive to drug with selective effects on noradrenergic transmission.¹⁹ Increasing swimming without significant increase in climbing like serotonergic agents predicts sedative effect of Ginger without stimulatory effect on loco-motor activity.

Zingiber officinale (150 and 300 mg/kg) showed significant decreased in immobility time ($p < 0.05$) in the dose dependent manner as compared to control mice. The extract at a dose of 300mg/kg body weight is found to be effective nearly similar to that of standard drug fluoxetine. The immobility is thought to reflect failure of persistence in escape directed behaviour. However, true mechanism of antidepressant effect of the Ginger rhizome is still unknown but behavioral parameters in forced swimming test confirmed potential antidepressant effect as serotonergic agents.

Growing evidence suggests that many traditional chemotherapeutic agents may be used alone or in combination with opioids to treat pain. Ginger (*Z. officinale* Roscoe), a well-known spice plant, has been used traditionally in the treatment of a wide variety of ailments, including arthritis, rheumatic disorders, and muscular pain. Our results showed that extract of *Zingiber officinale* (100, and 200 mg/kg, p.o.) significantly suppressed the acetic acid-induced writhing response in a dose-dependent manner in mice ($P < 0.05$) as compared to the vehicle control. Maximum inhibition of *Zingiber officinale* was observed at the dose of 200 mg/kg which is found to be effective nearly similar to standard drug, indomethacine.

It has been documented that ginger exhibits Ca^{2+} channel blocking activity.^{20, 21} Recently Ozgoli *et*

al.,²² have reported that ginger was as effective as mefenamic acid and ibuprofen in relieving pain in women with primary dysmenorrhea. Administration of ginger has a prophylactic effect in migraine headache without any side effects.²³

Furthermore, it has been documented that ginger extract produces significant analgesia against thermally (hot-plate test) induced nociceptive pain.²⁴ Apart from the crude extract, some of the known constituents of ginger (6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol) display a vasodilator effect through a combination of nitric oxide-releasing and calcium antagonist mechanisms.²⁵ Several lines of evidence indicate that nociception is related to the intraneuronal Ca^{2+} level. The lowering of the neuronal Ca^{2+} level induces analgesia.

It has been shown that gingerol constituents of ginger are relatively potent and efficacious agonists of the vanilloid 1 receptors.²⁶ Recent findings have demonstrated that administration of vanilloid compounds through alternative routes (injection of the agent into the epidural or subarachnoid space) produces highly effective antinociception.²⁷ Therefore, gingerols as potent vanilloid 1 receptor agonists also serve to explain the observed analgesic effect of ginger in this study.

Descending pain inhibitory circuits contribute to the control of spinal transmission of nociceptive information. Descending inhibitory circuits include the neuronal connections between the ventrolateral periaqueductal gray and rostral ventral medulla, which in turn project to the spinal cord dorsal horn lamina. There is a report indicating that vanilloid 1 receptor agonists and m-receptor agonists, when co-administered into the ventrolateral periaqueductal gray at nonanalgesic doses, produce an antinociceptive effect in nociceptive thermal tests.²⁸ We assume that such interaction is involved in the antinociceptive effect of ginger. However, this issue needs to be clarified by further studies.

CONCLUSION: From the above study we can conclude that the plant extract of *Zingiber officinale* showed significant antidepressant activity in forced swim test model and significant antinociceptive effect in acetic acid induced

writhing, thus making it a novel promising medicinal plant with diverse effect on central and peripheral nervous system with minimum side effects.

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CONFLICT OF INTEREST: No conflict of interest.

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