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EVALUATION OF ANALGESIC ACTIVITY OF METHANOL EXTRACTS OF *ZINGIBER OFFICINALE* IN MALE WISTAR RATS

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Abstract:

Zingiber officinale Leaves is a common weed occurring throughout the globe. In traditional medicine its decoction has been used for treatment of many infectious and degenerative diseases. This work was therefore designed to assess the phytochemical constitution of Zingiber officinale Leaves extracts and to evaluate their analgesic activity in rats. Fresh and crushed fruits of Zingiber officinale Leaves were collected and then extracted with ethanol. The ethanolic extract at the doses of 100 mg/kg, 200 mg/kg body weight was subjected to evaluation of analgesic activities in experimental animal models. Analgesic activity was evaluated by Hot-plate and tail –flick method in albino Wistar rats; Diclofenac sodium was employed as reference drugs for analgesic studies, respectively. In the present study, the ethanolic extract of Zingiber officinale Leaves demonstrated significant analgesic activities in the tested models.

Keywords: *Zingiber officinale, Hot-plate, Tail-flick model and Analgesic activity.*

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INTRODUCTION:

Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents (e.g. bacterial infection, physical trauma, chemicals or any other phenomenon) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair [1]. Inflammatory processes are required for immune surveillance, optimal repair, and regeneration after injury [2]. The inflammatory process protects our body from diseases by releasing cells and mediators that combat foreign substances and prevent infection [3]. However, sustained, excessive or inappropriate inflammation is the cause of numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease [4].

Inflammation is a major component of the damage caused by autoimmune diseases, and is a fundamental contributor of various infectious and non-infectious diseases such as cancer, diabetes, cardiovascular disease, rheumatoid arthritis, Alzheimer's and arteriosclerosis. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever [5, 6].

Inflammation is a complex pathophysiological process mediated by a variety of signalling molecules produced by leucocytes, macrophages and mast cells undergoing various cellular responses such as phagocytic uptake, and the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2) and tumour necrosis factor (TNF)-α [7], that bring about edema formation as a result of extravasation of fluid and proteins and accumulation of leucocytes at the inflammatory site [8]. In addition, it is broadly accepted that cytokines, produced by either immune or central nervous system cells, might directly sensitize the peripheral nociceptors [9].

Inflammation is an important cellular response triggered by various mechanical, chemical or immunological stress factors and it is regulated by a delicate balance between local factors that finally determine the outcome of the disease process: progression or resolution. The inflammatory response is a complex and highly regulated sequence of events that start with an initial production of pro-inflammatory mediators that recruit professional inflammatory cells to the site of injury to clear the offending trigger [10]. This is followed by an anti-inflammatory phase, in which resident tissue cells may acquire the potential for protecting themselves from further activation and injury. More recently, inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality" or "the reaction to injury of the living microcirculation and related tissues" [11].

Although, in ancient times inflammation was recognised as being part of the healing process, up to the end of the 19th century, inflammation was viewed as being an undesirable response that was harmful to the host. Based on visual observation, the ancients characterised inflammation by five cardinal signs, namely redness (rubor), swelling (tumour), heat (calor; only applicable to the body extremities), pain (dolor) and loss of function (functio laesa). The first four of these signs named by Celsus in ancient Rome (30-38 B.C.) and the last by Galen (A.D. 130-200) [12].

The classical description of inflammation accounts for the visual changes seen. The sensation of heat is caused by the increased movement of blood through dilated vessels into the environmentally cooled extremities. Redness is due to the additional number of erythrocytes passing through the area. Swelling (edema) is the result of increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues, infiltration of cells into the damaged area, and in prolonged inflammatory responses deposition of connective tissue. Pain is due to the direct effects of mediators, either from initial damage or that resulting of sensory nerves due to oedema. Loss of function refers to either simple loss of mobility in a joint, due tothe oedema and pain, or to the replacement of functional cells with scar tissue.

Inflammatory process has two phases: acute and chronic. Acute and chronic inflammations are known to be complicated processes induced by several different classes of chemical mediators, e.g. prostaglandins, leukotrienes and platelet-activating factor, etc. Anti inflammatory agents exert their effect through a spectrum of different modes of action. [13]

Acute inflammatory response is characterized by an increase in vascular permeability and cellular infiltration leading to oedema formation as a result of extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site for short time [14].

Chronic inflammation is the reaction arising when the acute response is insufficient to eliminate the pro-inflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and infiltration of neutrophils with exudation of fluid. It occurs by means of development of proliferative cells which can either spread or form granuloma. Chronic inflammation may also occur due to the

persistence of infection or antigen, recurring tissue injury, or a failure of endogenous anti-inflammatory mechanisms.

Chronic (or acute) inflammation is a multiple process mediated by activating inflammatory or immune cells [15], among which macrophages play a central role in managing many different immunopathological phenomena including the overproduction of proinflammatory cytokines and inflammatory mediators, generated by activated COX-2[16]. Under inflammatory conditions, immune cells are also stimulated by adhesion molecule activation signals in order to enhance the migration capacity to inflamed tissue and finally to form heterotypic cell clustering between the immune cells, endothelial cells and inflamed cells [17].

Macrophages in the inflammatory reaction initially requires an interaction between surface receptors such as Toll-like receptors (TLR) and stimuli [18], and subsequent up-regulation of intracellular signalling events mediated by enzymes such as phosphoinositide 3-kinases (PI3K) and mitogen activated protein kinases (MAPKs) as well as transcription factors (e.g., nuclear factor [NF]-κB and activator protein [AP]-1) (Sekine et al., 2006). Overall, these events lead macrophages to express pro-inflammatory genes such as inducible NO synthase (iNOS) and cyclooxygenase (COX)-2. Because large amounts of macrophage-derived inflammatory mediators can cause collateral or severe damage such as septic shock, rheumatoid arthritis and arteriosclerosis [19], the effective blockade of these inflammatory responses is an important therapeutic target. Inflammatory diseases are a major cause of morbidity of the work force throughout the world. These have been called the "King of Human Miseries" [20].

Inflammatory diseases

Inflammation is a physiological response of a body to stimuli, including infections and tissue injury. However, excessive or persistent inflammation causes a variety of pathological conditions [21]. As the primary interface between the body and the external environment, the skin provides the first line of defense against traumatic injury and invasion by microbial pathogens. In addition to its properties as a physical barrier, the skin has many active defence mechanisms [22] and regulation of these mechanisms is crucial, as inappropriate or misdirected immune activity is implicated in the pathogenesis of a large variety of inflammatory skin disorders.

Many degenerative diseases such as rheumatoid arthritis, shoulder tendonitis, gouty arthritis, polymyalgia rheumatica, heart disease, asthma, and inflammatory bowel disease are often associated with inflammatory processes [23].

Standard drugs for inflammation and side effects

Many steroids, specifically glucocorticoids and Mineralocorticoids reduce inflammation or swelling by binding to corticoid receptors. These drugs are often referred to as corticosteroids. Long-term corticosteroids use has several severe side effects eg. hyperglycemia, insulin resistance, diabetes mellitus, osteoporosis, anxiety effects etc. [24]

There are over 50 different NSAIDs available [25] and they can be divided into different groups based on their chemical structure, pharmacokinetics and selectivity towards Cox-1 or Cox-2 [26]. NSAIDs can be classified broadly into two types based on their chemical structure. Most NSAIDs are carboxylic acids; but a few, most noticeably phenylbutazones, are enolic acids. Carboxylic acid containing drugs include salicylate derivatives (eg. aspirin), carbocyclic and hetrocyclic acid derivatives (eg. indomethacin), fenamic acid derivatives (eg. Ibuprofen, ketoprofen, fenbufen, flurbiprofen, suprofen and naproxen) and phenyl acetic acid derivatives (eg. diclofenac, aceclofenac, etc.). Enolic acid containing drugs include oxicam derivatives (eg. piroxicam, tenoxicam and meloxicam) and pyrazoles (eg. phenylbutazone and oxyphenbutazone). Non acidic group compounds include nabumenton.

Most of the NSAIDs have three major types of action: [27]

1) Anti-inflammatory action for treating several conditions including rheumatoid arthritis, osteoarthritis, musculoskeletal disorders and pericarditis.

2) Analgesia for treating pain of mild to moderate intensity. Their maximum therapeutic efficiency is much lower than that of the opioids, but they do not cause dependence.

3) Antipyretic action, which mediates by the release of endogenous pyrogen from monocytes and macrophages in the presence of infection or inflammation.

Non-steroidal anti-inflammatory drugs (NSAIDs) typically relieve inflammation and associated pain by inhibiting cyclooxygenase enzymes involved in the production of prostaglandins. These enzymes exist in two isoforms (COX-1 and COX-2) coded by distinct genes on different chromosomes[28].

6. MATERIALS AND METHODS:

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including

selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

6.1 DRUGS AND CHEMICALS

Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Table No: 6.1 Drugs and Chemicals

S.No	*Materials*	*Company Name*
1.	Ethanol	Honeywell
2.	Diclofenac	IPCA Laboratories.

6.2. INSTRUMENTS

Following instruments were required for the study:

Table No: 6.2- List of Instruments used for study

Name of the instrument	*Source*
Centrifuge	Dolphin
Digital weighing balance	Horizon
Heating mantle	ASGI®
Plethysmometer	Dolphin
HotPlate	Johnson and Johnson, china
Analgesiometer	Dolphin
Refrigerator	Videocon
Soxhlet extractor	ASGI®
Condenser	ASGI®
Burette stand	Dolphin
Round bottom flask	ASGI® , Amar
Mixer	Videocon
Oven	ASGI®
Water bath	ASGI®
Stirrer/glass rod	ASGI®
Watch glass	ASGI®
Whatmann filter paper	Manipore microproducts, Ghaizabad.
Butter paper	ASGI®
Spatula	ASGI®
Rubber pipes	ASGI®

6.3. EXPERIMENTAL ANIMALS

Healthy adult albino Wistar rats weighing 200-250grams of either sex were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. They were fasted overnight before the day of experiment, after 72hours of fasting from the day of Alloxan introduction. Animals were housed within the departmental animal house and the room temperature was maintained at 27°C. Animal studies had approval of IAEC.

6.4. PLANT MATERIAL COLLECTION

The leaves of *Zingiber officinale* was collected from the local market. The plant root material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

6.5. PREPARATION OF PLANT EXTRACTS:

6.5.1 Preparation of ethanolic Extract:

Fresh Leaves of *Zingiber officinale* were collected and washed under tap water. The leaf extract used was prepared by taking 50gms of finely cut roots into 250ml beaker containing 200ml of ethanol. The contents were mixed well and then the mixture was boiled up to 50-60^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

6.6. SELECTION OF DOSE FOR ANIMAL STUDY

The dose considered for the experiment on rats was obtained from conversion of human dose of *Zingiber officinale Leaves* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats. Hence the calculated dose for the rats (considering human dose 5 g/kg) is 100 and 200 mg/kg. Acute toxicity was done at dose of 2000 mg/kg body weight.

6.7. PHARMACOLOGICAL EVALUATION

Preparation of extracts:

The ethanolic extracts of *Zingiber officinale Leaves* suspended in water in presence of 3%v/v Tween-20 solution.

All the drugs were administered orally for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

6.8. ACUTE ORAL TOXICITY:

The acute oral toxicity of ethanolic extracts of *Zingiber officinale Leaves* was determined by using Albino Wistar rats (200-250g) which were maintained under standard conditions. The animals were fasted 12 hours prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed up to 7days for their mortality, behavioral and neurological profiles.

6.9. ASSESSMENT OF ANALGESIC ACTIVITY:

Table: Group Classification:

Group	Treatment	Dose(mg/kg)
Group 1	Normal control received distilled water	------
Group 2	Standard group received Diclofenac	10ml/kg
Group 3	Ethanolic extract of *Zingiber officinale Leaves*	100
Group 4	Ethanolic extract of *Zingiber officinale Leaves*	200

- **HOT-PLATE METHOD:**

Procedure:

Animals were grouped and divided randomly into four groups of four and each was fasted to overnight. Take the basal reaction-time by observing hind paw licking or jumping response in animals when placed on the hot-plate maintained at constant temperature (55^{0}C). Normally animals showed such response in 6-8 sec. a cut-off period of 15sec i.e observed to avoid damage to the paws. Inject the standard drug to group-2 and test drugs to group-3&4 and noted the reaction time of animals on the hot plate at 15, 30, 60 and 120min after the drug administration. Calculated the percentage increase in reaction time at each time interval.

- **TAIL-FLICK METHOD:**

Procedure:

Animals were grouped and divided randomly into four groups of four and each was fasted to overnight. The animals were screened for sensitivity test by placing the tip of the tail on the radiant heat source. Any animals that held to withdraw its tail in 5 second was rejected from the study. Analgesia was assessed with a tail flick apparatus (Analgesiometer). The basal reaction time was measured initially and another set of four measures were taken as 15, 30, 45 and 60 minutes interval and the reaction of the animals considered as the post – drug reaction time. A cut-off period of 10sec. was observed to prevent tissue damage of the animals.

6.10. STATISTICAL ANALYSIS:

The values were expressed as mean ± SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparison had made. i.e.

1. Normal control Vs All treated groups.

Differences between groups were considered significant at P<0.001 and $P < 0.05$ levels.

8. RESULTS:

7.1 ACUTE TOXICITY STUDIES:

Acute toxicity studies revealed that the ethanolic extracts of *Zingiber officinale Leaves* were safe up to 1000 mg/kg of body weight and approximate LD 50 is more than 2000 mg/kg. No lethality or any

toxic reactions was observed up to the end of the study period.

7.2 EVALUATION OF ANALGESIC ACTIVITY

7.2.1 HOT PLATE METHOD:

The analgesic activity of EEZO was assessed using Hot plate method in Swiss albino rats were illustrated in Table. EEZO showed significant analgesic activity at 100 and 200 mg/kg, i.p. Analgesic activity was comparable with the standard drug Diclofenac. Both doses have showed maximum analgesic activity at reaction time is 12 and 5 sec respectively and the standard drug Diclofenac reaction time is 10 sec.

Table No: 7.8- Effect of extracts of *Zingiber officinale* Leaves on Analgesic activity

Groups	Dose	Basal reaction time(sec)		After drug administration(sec)							
		0min		15min		30min		60min		120min	
		Paw licking(P)	Jumping(J)	P	J	P	J	P	J	P	J
Control	10	-	9	4	6	5	3	3	-	5	-
Standard	40	9	-	10	-	6	-	14	-	-	10
EEZO	100	14	-	5	-	12	-	8	-	12	-
EEZO	200	12	10	4	10	-	10	-	12	-	5

The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

7.2.2 TAIL-FLICK METHOD:

The analgesic activity of EEZO was assessed using Tail-Flick method in Swiss albino rats were illustrated in Table. EEZO showed significant analgesic activity at 100 and 200 mg/kg, i.p. Analgesic activity was comparable with the standard drug Diclofenac. Both two doses showed maximum analgesic activity at reaction time is 14 and 12 sec respectively and the standard drug Diclofenac reaction time is 13 sec.

S.No	Treatment	Dose (mg/kg)	Reaction Time(sec)				
			30min	45min	60min	75min	90min
1.	control	-	5.10±0.53	6.01±0.12	5.98±0.10	6.10±0.20	6.10±0.03
2.	standard	10	8.30±0.11	12.13±0.05	12.20±0.14	11.13±0.14	11.35±0.26
3.	EEZO	100	9.63±0.26	9.23±0.10	11.30±0.05	10.94±0.09	11.50±0.01
4.	EEZO	200	8.89±0.02	9.99±0.21	11.09±0.23	11.63±0.02	11.02±014

The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

8. DISCUSSION:

8.1 ANALGESIC ACTIVITY

8.1.1 HOTPLATE METHOD:

The extracts increased reaction latency to thermal pain induced by the hot plate method in rats, which is a specific central antinociceptive test. Inhibition of histamine or kinin pathway may reduce pain. The results of the present study also showed that extract exhibited a comparable magnitude of antinociceptive activity in hot plate method of pain which suggested that the phytochemical constituents are responsible for the analgesic effect. The results of the present study indicated that the ethanolic extracts of *Zingiber officinale Leaves* might contain constituents capable of relieving or modifying responses to pain caused by either thermal or chemical stimulation of the nociceptors mediated by both central and peripheral mechanisms.

8.1.2 TAIL FLICKMETHOD:

The extracts showed significant analgesic activity at all tested dose levels. In tail flick method, the ethanolic extracts of *Zingiber officinale Leaves* at a dose of 100 and 200 mg/kg showed significant activity. The results showed significant analgesic activity against thermal stimuli. The analgesic studies revealed that the ethanolic extract of *Zingiber officinale Leaves* exhibited potent analgesic (central analgesic activity) effect against thermal noxious stimuli and also revealed that the extract shows dose dependent analgesic effect.

9. CONCLUSION:

Zingiber officinale Leaves is a plant and it has anti-diabetic, anti-bacterial, anti-microbial, anti-fungal, anti-oxidant and CNS activities. Among these studies it could be concluded that fruit of *Zingiber officinale Leaves* have shown great potential of Analgesic activity. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific drugs.

In the Present study it has concluded that ethanolic extracts of *Zingiber officinale Leaves* have an analgesic activity in both Hotplate and Tail-flick method in rats. This extract has showed that increase mean latency to thermal pain. The presence of some chemical constituents contains a capable of relieving or modifying responses to pain caused by inhibition of histamine or kinnin pathway.

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