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Pharmacological Screening of Biological Activities of Ginger Juice and Ethanolic Extract of Zingiber Officinale

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ABSTRACT
The present study focused on pharmacological screening of biological activities like analgesic, anxiolytic, muscle relaxant and anti-mitotic activity of ginger juice and ethanolic extract of zingiber officinale. Ginger juice and ethanolic extract of ginger were prepared using soxhlet apparatus and screened for phytochemical analysis showed the presence of alkaloids, carbohydrates, phenolic compounds, gum, mucilage, flavonoids, volatile oil, whereas ethanolic extract of ginger showed presence of terpenoids but not ginger juice.
The dried form of extract was dissolved in 0.9% saline and injected to albino mice which were grouped into control, standard and two test groups. Ethanolic extract of ginger showed good analgesic, muscle relaxant and anti-mitotic activity but not anxiolytic activity while ginger juice showed good muscle relaxant and anti-mitotic activity but not analgesic and anxiolytic activity.
Diclofenac sodium, Pentazocin, Diazepam, Methotrexate are used as standard references.
KEY WORDS: Zingiber officinale, analgesic activity, anxiolytic activity, skeletal muscle relaxant activity, anti-mitotic activity.

I. INTRODUCTION

Pharmacological screening: It is the method of investigation or measure of pharmacological activities of a new or chemically undefined substances, function of endogenous mediators.[1]

Biological activity:
It describes the beneficial effects of a drug on living matter. It play's a crucial role since it suggests uses of compounds in medical applications.[2] The biological activities that were screened in the present study includes –

1) Analgesic
2) Muscle relaxant
3) Anxiolytic
4) Anti-mitotic activity.

1.1 ANALGESIC ACTIVITY

Pain is an unpleasant sensory emotional experience associated with actual or potential tissue damage or described in terms of such damage. It is an alert mechanism to prevent further tissue injury.
The experience of pain in humans can be classified temporarily as acute or chronic. Pain that lasts a long time is called chronic or persistant, and pain that resolves quickly is called acute.
The three classes of pain

- Nociceptive pain,
- Inflammatory pain which is associated with tissue damage and the infiltration of immune cells,
- Pathological pain which is a disease state caused by damage to nervous system or by its abnormal function (tension type headache, fibromyalgia)

Nociceptive pain is caused by ongoing activation of A-gamma and C- nociceptors in response to noxious stimulus (inflammation). It can be further classified into visceral pain(deep cramping sensation associated with referred pain), superficial somatic pain (skin: well localized sharp, pricking sensation) and deep somatic pain(muscle, joint capsules and bone)

Neuropathic pain is caused by damage or disease affecting any part of the nervous system involved in bodily feelings (somatosensory system). This leads to abnormal processing of sensory information by the nervous system. Neuropathic pain can be classified as peripheral and central and can originate from nerve injury following a wide array of events including direct trauma to nerves, inflammation/neuritis, diabetis, tumours and primary neurological diseases.

CLASSIFICATION Analgesics are divided into two groups:-
A. Opiod / Narcotic / Morphin like analgesics
B. Non opiod / Non narcotic / Aspirin like / Antipyretic/Anti inflammatory analgesics

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NON-OPIOID ANALGESICS

Non-opioid analgesics are pain medications for mild to moderate pain. Non-opioid analgesics include NSAIDs (nonsteroidal anti-inflammatory drugs) such as ibuprofen, as well as other analgesics such as aspirin. Non-opioid analgesics may be short-acting or long-acting pain medications. They may be taken alone for pain management or they may also be taken in combination with opioids to relieve moderate to severe pain. These are classified as –

1) Salicylic acid derivatives - Acetyl salicylic acid.
2) Acetic acid derivatives – Diclofenac, indomethacin.
3) Propionic acid derivatives – Ibuprofen, ketoprofen.
4) Non-acids – Paracetamol.

Side effects of Non-opioids – They cause upper gastrointestinal complications, including ulcers, perforation and bleeding.[3]

1.2 ANXIOLYTIC ACTIVITY

Anxiety is a diffuse state of mood and has been considered to be an unpleasant affective experience marked by a significant degree of apprehensiveness about the potential appearance of future aversive or harmful events.

Human anxiety is defined as a feeling of apprehend generalized anxiey disorder(GAD) with considerable degree of disability.[4]

TYPES OF ANXIETY-

There are several recognized types of anxiety disorders, including;

Panic disorder:

People with this condition have the feelings of terror that strike suddenly and repeatedly with no warning.

Obsessive-compulsive disorder (OCD):

People with OCD are plagued by constant thoughts or fears that cause them to perform certain rituals or routines. The disturbing thoughts are called obsessions, and the rituals are called compulsions.

Post-traumatic stress disorder (PTSD):

PTSD is a condition that can develop following a traumatic and/or terrifying events, such as a sexual or physical assault, the unexpected death of a loved one, or a natural disasters.

Social anxiety disorder:

Also called social phobia, social anxiety disorder involves overwhelming worry and self consciousness about everyday social situations.

Specific phobias:

A specific phobia is an intense fear of a specific object or situation, such as snakes, heights, or flying.

Generalized anxiety disorder:

This disorder involves excessive, unrealistic worry and tension,even if there is little or nothing to provoke the anxiety.[5]

SYMPTOMS- Symptoms include:

- Feelings of panic, fear, uneasiness.
- Repeated thoughts or flashbacks of traumatic experiences.
- Nightmares.
- Palpitations.
- Dry mouth.[6]

TREATMENT OF ANXIETY

Initially this psychological disorder was treated using the traditional anxiolytic/hypnotic agents. Newer anticonvulsant and antipsychotic drugs are also used in the treatment of some anxiety disorders. Benzodiazepines can start working more quickly than antidepressants. The ones used to treat anxiety disorders include:

Benzodiazepines:

- Alprazolam (Xanax)
- Bromazepam (Lexotan)
- Clonazepam (Klonopin)
- Diazepam (Valium)

Benzodiazepines affect the gamma-amino butyric acid(GABA) receptors of the brain. This action results in slowing of the central nervous system(CNS), including state of relaxation.

1.3 SKELETAL MUSCLE RELAXANTS

A **muscle relaxant** is a drug that affects skeletal muscle function and decreases the muscle tone. It may be used to alleviate symptoms such as muscle spasms, pain, and hyperreflexia. The term "muscle relaxant" is used to refer to two major therapeutic groups:

1.Neuromuscular blockers
2. Spasmolytics.

Neuromuscular blockers act by interfering with transmission at the neuromuscular end plate and

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have no central nervous system (CNS) activity. They are often used during surgical procedures and in intensive care and emergency medicine to cause temporary paralysis.[8]

These are of two types

A. Nondepolarizing agents

B. Depolarizing agents

A. Neuromuscular non-depolarizing agent is a form of neuromuscular blocker that does not depolarize the motor end plate. . They act by competitively blocking the binding of ACh to its receptors, and in some cases, they also directly block the ionotropic activity of the ACh receptors.[9]

- The quaternary ammonium muscle relaxants belong to this class.

B. Depolarizing neuromuscular blocking agent is a form of neuromuscular blocker that depolarizes the motor end plate.[10]

- An example is succinylcholine.

B. Depolarizing blocking agents work by depolarizing the plasma membrane of the muscle fiber, similar to acetylcholine. However, these agents are more resistant to degradation by acetylcholinesterase, the enzyme responsible for degrading acetylcholine, and can thus more persistently depolarize the muscle fibers.[10]

There are two phases to the depolarizing block. During phase I (depolarizing phase), they cause muscular fasciculations (muscle twitches) while they are depolarizing the muscle fibers. Eventually, after sufficient depolarization has occurred, phase II (desensitizing phase) sets in and the muscle is no longer responsive to acetylcholine released by the motoneurons. At this point, full neuromuscular block has been achie

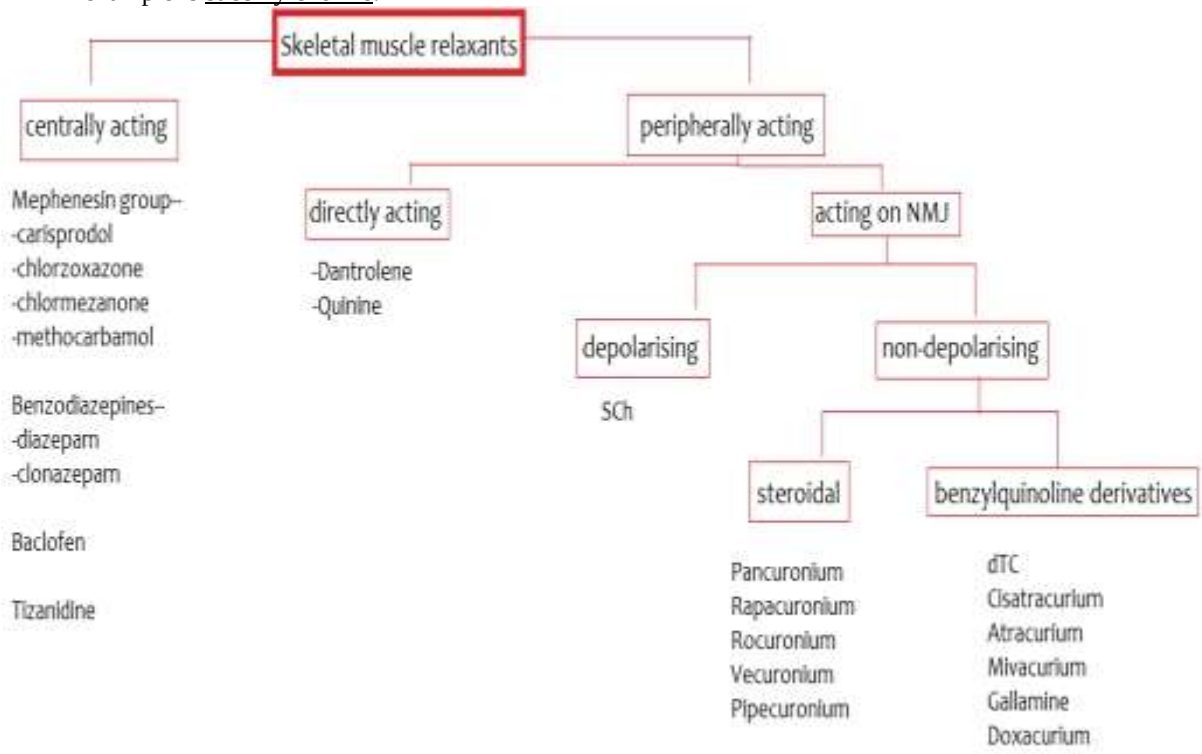

Figure 2.3.1 Classification of skeletal muscle relaxants.

Common side effects of skeletal muscle relaxants:

- Weakness or fatigue
- Dizziness
- Dry mouth
- Sedation

1.4 ANTIMITOTIC ACTIVITY

Cancer refers to abnormal growth or malignant tumors and is characterized by uncontrolled proliferation of cells despite restriction of nutrients and space.[13] Cancer cells have unlimited replicative potential via the upregulation of telomerase (a specialized

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deoxyribonucleic acid (DNA) polymerase) expression that counters telomerase erosion.[14]

Antimitotic drugs inhibit polymerization dynamics of microtubules (paclitaxel and vinblastine) by activating the spindle assembly checkpoint (SAC) blocking transition from metaphase to anaphase. Subsequently, cells undergo mitotic arrest.

Drugs that act on microtubules can be divided into two groups according to their mechanism of action as either

A. Microtubule-destabilizing agents
B. Microtubule-stabilizing agents.[15]

Destabilizing drugs inhibit the polymerization of microtubules when administered at high concentration.[16] Most destabilizing drugs bind to either the vinca domain or taxoid-binding domain.[17] Those that bind to the vinca domain found in the interface between β- and α-tubulin (called vinca alkaloids) include vinflunine, vincristine, vinorelbine, vindesine. Those that bind to the colchicine domain include cryptophycins, dolastatins and combretastatin-A4.[18]

Drugs that enhance microtubule polymerization when administered at high concentrations, stabilize microtubules and prevent Ca^{2+}- or cold-induced depolymerization, and subsequent disassembly, include eribulin, spongistatin, rhizoxin, maytansinoids second- and third-generation taxanes, epothilones, ixabepilone and many others.[19,20] Taxanes, epothilones and many others belonging to this group bind to the inner surface of the microtubule at a taxoid-binding site on β-tubulin.[21]

Classes of Antimitotic drugs

Taxens : Paclitaxel, Cabazitaxel
Epothilones : Epothilones A and B, Ixabepilone
Vinca alkaloids: Vincristine, Vinorelbin, Vindesin
Microtubule targeting estrogen derivatives: Fulvestran
2-Methoxyestradiol and in silico-designed analogs

Common side effects of Antimitotic drugs:

- Nausea and Vomiting
- Neurotoxicity
- Diarrhoea
- Peripheral neuropathy
- Myelosuppression
- Arthralgias
- Skin reaction

II. PLAN OF WORK

- Collection of Zingiber officinale rhizomes.
- Getting authentification of rhizome from botany department of O.U.
- Preparation of ginger juice.
- Preperation of ethanolic extract of ginger by soxhlet extraction.
- Phytochemical screening of ginger juice and ethanolic extract of ginger.
- Evaluation of biological activities by using wrister albino mice.

1.Analgesic activity:
1. Centrally - Eddy's hot plate method, Tail flick response.
2. Peripherally – Acetic acid induced writhing response.

2.Anxiety activity:
1. Elevated plus maze method.

3.Skeletal muscle relaxant activity:
1. Rota rod method.

4.Anti-mitotic activity
1. Seed germination method.

III. MATERIALS AND METHODS

The rhizomes of Zingiber officinale were purchased from market. Authentication of ginger rhizome has been done in the Department of Pharmacognosy, University of Osmania.

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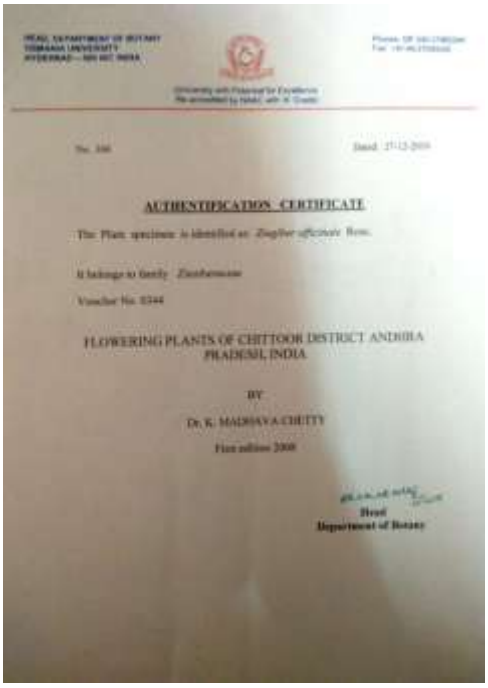
AUTHENTIFICATION CERTIFICATE

FLOWERING PLANTS OF CHITTOOR DISTRICT ANDHRA PRADESH, INDIA

BY

Dr. K. MADHAVA CHETTY

Figure 3.0 Authentication certificate of ginger

3.1 Experimental Animals

Albino mice weighing 18-25 g of either sex were used for the study. The animals were procured and housed in the animal house maintained under standard hygienic conditions, humidity (60 ± 10%) with 12 hour day and night cycle, with food and water. The study was carried out as per CPCSEA (Committee for the purpose of Control and Supervision of Experiments on Animals).

3.2 GROUPING OF ANIMALS:

- Animals were divided into 4 groups.
- Each group containing 6 animals.
- First group of animals were administered with saline.
- Second group of animals were administered with standard Drug.
- Third group of animals were administered with ginger juice.
- Fourth group of animals were administered with ethanolic extract of ginger.

3.3 DRUGS AND CHEMICALS:

- Diclofenac sodium
- Pentazocin
- Methotrexate
- Diazepam
- Absolute ethanol
- Ginger juice
- Ethanolic extract of ginger

3.4PREPARATION OF GINGER JUICE

- The commercially available ginger was obtained from the local market. It was confirmed from the botanist that it was Zingiber officinale. The rhizome of ginger after cleaning and scrapping the superficial skin was cut into small pieces.
- With the help of mixer-grinder the pieces were made into paste. The paste was taken on a white clean cloth and the liquid was squeezed out. The juice so obtained was used in the experiments.
- The stock of juice was kept in a refrigerator for maximum period of 15 days and the required quantity was used for the experiments after removing particulate matter from it. 100gm ginger rhizome yielded about 250ml juice and is filtered.
- The residue obtained is dried at room temperature yielded about 7 grams.[37]

3.5 PREPARATION OF ETHANOLIC EXTRACT OF GINGER

Method of extraction:
Solvent: Absolute ethanol (95.5%)
Method: Soxhlet extraction.

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Procedure:

- The rhizome of ginger after cleaning and scrapping the superficial skin was cut into 5mm pieces which are to be extracted were placed in the thimble.
- The thimble was loaded into the main chamber of the soxhlet extractor. The extraction solvent to be used was placed in the distillation flask. The solvent was heated to reflux. The solvent vapour travels up the distillation arm, and floods into the chamber housing the thimble of solid.
- The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent.
- When the Soxhlet chamber is almost full, the chamber was emptied by the siphon. The solvent was returned to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle may be allowed to repeat many times, over hours or days.
- During each cycle, a portion of the non-volatile compound dissolved in the solvent. After many cycles the desired compound was concentrated in the distillation flask.[38]

Figure 3.5.1 SOXHLET EXTRACTION APPARATUS

3.6 PRELIMINARY PHYTOCHEMICAL ANALYSIS:

Ethanolic extract of ginger and ginger juice were subjected to preliminary phytochemical analysis to test for the presence or absence of various phytoconstituents

IV. PHARMACOLOGICAL SCREENING AND INVIVO METHODS

4.1 EVALUATION METHODS: ANALGESIC ACTIVITY

4.1.1 EDDY'S HOT PLATE METHOD:

The paws of mice are highly sensitive to heat at temperatures which do not damage their skin. In this method heat was used as a source of pain. The mouse responds by jumping, withdrawing of paws (or) licking the paws.[39]

Methodology: Albino mice of either sex were selected which are weighing between 25-35 gm randomly divided into 4 groups each group containing six mice (n =6)

Group –I control
Group –II Pentazocine (10mg/kg) (Standard) S.C
Group –III Ethanolic extract of Ginger (50mg/kg) i.p
Group –IV Ginger juice (50mg/kg) i.p

The mice were weighed and numbered on tail and the basal reaction time was noted by observing hind paw licking (or) jump response

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(which ever appears first) in animals when placed at hot plate maintained at constant temperature (55°C). A cut off period of 15 sec was observed to avoid the damage to the paw. The Pentazocine 10mg/kg was injected subcutaneously and all other drugs were administered by i.p after basal reaction, the reaction is observed at 30, 60, 90 min, after injection of drugs.[40,41]

Figure 4.1.1.1 Eddy's hot plate apparatus

4.1.2 WRITHING TEST:

A variety of chemical agents have been used for producing pain. The Intraperitoneal (i.p). administration of noxious chemical substance to mice and cats produces peritoneal irritation, which elicits a writhing response. The response is unlearned and reflexive in nature. Each episode of writhing is characterized by internal rotation of the feet, sucking in the belly, elongation of the body, arching of the back, rolling on one side and remaining still, or turning around and circling the cage. Many chemical agents have been reported to produce writhing but acetic acid and phenyl benzoquinone are the two most commonly used irritants. However, the use of phenyl benzoquinone has been associated with problems of solubility, photosensitivity & auto-oxidation.

Methodology:

Albino mice (25-30gm) of either sex were used. The mice were divided into four groups with six mice in each group. All the mice were fasted for 18hr before experiment (preferably over night)

Group-I control
Group-II Diclofenac sodium (9mg/kg)
Group-III Ethanolic extract of Ginger (50mg/kg)
Group-IV Ginger juice (50mg/kg)

All the drugs were administered through i.p route. Thirty minutes after administration of standard and test sample. Mice were given 1% v/v solution of acetic acid i.p, the dose being 0.1ml/10gm body weight of mice. The writhing produced in these animals were counted for 10 minutes. The time taken for the onset of writhing was noted (Induction time). The number of writhing produced in standard group were compared with test groups.[42]

$$\%\text{Effectivity} = \frac{\text{Expt}}{\text{control}}$$

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4.1.3 TAIL FLICK RESPONSE:

Albino mice (25-30gm) of either sex were used. The mice were divided into four groups with six mice in each group. All the mice were fasted for 18hr before experiment (preferably over night)

Group-I control
Group-II Diclofenac sodium (9mg/kg)
Group-III Ethanolic extract of Ginger (50mg/kg)
Group-IV Ginger juice (50mg/kg)

Mice were held in a suitable restrainer with the tail protruding out. Radiant heat was applied over the tail on a single spot with the help of a suitable device. The time taken by the animal to withdraw the tail was taken as reaction time. In this method animals were selected by preliminary screening. Those showing variation of more than one second between two reaction times at 15minute interval or more than 3 seconds from the group were discarded. The cut off time period was 10-12 sec. The time response curves may be plotted or ED_{50} values calculated.[43]

ANXIOLYTIC ACTIVITY

4.1.4 ELEVATED PLUS MAZE METHOD:

The animals were weighed and numbered later divided them into 4 groups each consisting of 6 mice.
Group-I control
Group-II Diazepam (2mg
Group- III Ethanolic extract of Ginger (50mg/kg)
Group- IV Ginger juice (50mg/kg)

Animlas were individually placed in the centre of the maze, head facing towards open arm and stop watch was started then the following parameters for 5 minutes has been noted -

- First preference of mouse to open or enclosed arms.
- Number of entries in open and enclosed arms.
- Average time each animal spends in each arm.

(Average time = Total duration in arm/No. of entries)

- The latency was recorded before and after 30, 60 & 90 min following oral and i.p. administration of the test compounds & the standard drug respectively.
- Comparision of preference of the animal to open or enclosed arms, average time spent in open arm and number of entries in open arm in each group was done.[44]

Figure 4.1.4.1 Elevated plusmaze apparatus

MUSCLE RELAXANT ACTIVITY

4.1.5 ROTA ROD METHOD:

- Animals were weighed and numbered later divided into 4 groups each group consisting of 6 mice (n=6)

Group-I Control
Group-II Diazepam (2mg/kg)
Group-III Ethanolic extract of Ginger (50mg/kg)
Group-IV Ginger juice (50mg/kg)

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- Rota-rod was turned on. An appropriate speed (20-25rpm) is ideal.
- The animals were placed one by one on the rotating rod. The "fall off time" when the mice falls from the rotating rod was noted.
- The latency was recorded before and after 30, 60 & 90 min following oral and i.p. administration of the test compounds & the standard drug respectively.
- Comparision of the fall off time of animals before and after administration of drugs was done.[45]

Figure 4.1.5.1 Rota rod apparatus

ANTI-MITOTIC ACTIVITY

4.1.6 Seed Germination Assay

Seed germination assay was evaluated by using green gram seeds (Phaseolus radiatus).

Experimental design:

Green gram seeds were divided in to 4 groups and each group containing 10 green gram seeds.

Procedure:

Green gram seeds were collected from the local market and each seed weighed individually.50 mg/ml concentration of seed coat extract was prepared. Methotrexate was used as standard drug. Distilled water was used as a control. Ginger juice and ethanolic extract of ginger were taken as test. Equal weights of seeds were added in the sample vials. The vials were left at room temperature for 24hrs for imbibition of water. After 24hrs drug treated seeds were dried on tissue paper and weighed. The time of sprouting was extended to 72hrs and photographs were taken.[47]

$$\text{Percentage of inhibition} = \frac{[\text{(wt D- wt S)}]}{[\text{(wt D- wt M)}]} \times 100$$

Where, wt D= Seed weight in distilled water
wt E= Seed weight in extract sample
wt M= Seed weight in methotrexate

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Figure 4.1.6.1 Seed germination assay

V. RESULTS

5.1 Preliminary Phytochemical Studies Of Ginger Juice And Ethanolic Extract Of Ginger (Zingiber officinale)

The revealed results of the preliminary preliminary phytochemical screening of the Ginger juice and Ethanolic extract of Ginger. Results were shown below.

Phytochemicals	**Inference**	
	Ginger juice	Ethanolic extract of Ginger
Alkaloids	Present	Present
Carbohydrates	Present	Present
Glycosides	Absent	Absent
Phenolic compounds	Present	Present
Tannins	Absent	Absent
Protein and Amino acids	Absent	Absent
Saponin glycosides	Absent	Absent
Gum and Mucilage	Present	Present
Sterols	Absent	Absent
Flavanoids	Present	Present
Terpenoids	Absent	Present
Volatile oil	Present	Present

Table – 5.1.1 Results of preliminary phytochemical studies.

5.2 Analgesic activity

S.no	Groups	Time to lick or jump in sec			
		0min	30min	60min	90min
1	Group-I (Control)	3.66±1.52	3.5±1***	3.66±1.52***	3.5±1.5***
2	Group-II (Pentazocine 10mg/kg)	3.66±1.52	11.3±1.15***	14±1***	13±1***
3	Group-III Ethanolic extract of ginger (50mg/kg)	5.33±0.5	13±2***	11.33±1.52***	14±1***

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4	Group-IV Ginger juice (50mg/kg)	5.66±0.5	7.33±1.52^{ns}	6.66±1.52^{ns}	6.33±1.52^{ns}

Table 5.2.1 Results of eddy's hot plate method:

Data was expressed as mean ± S.D., n=6
*****P < 0.05, **P <0.01, ***P < 0.001 versus control ns= not significant.

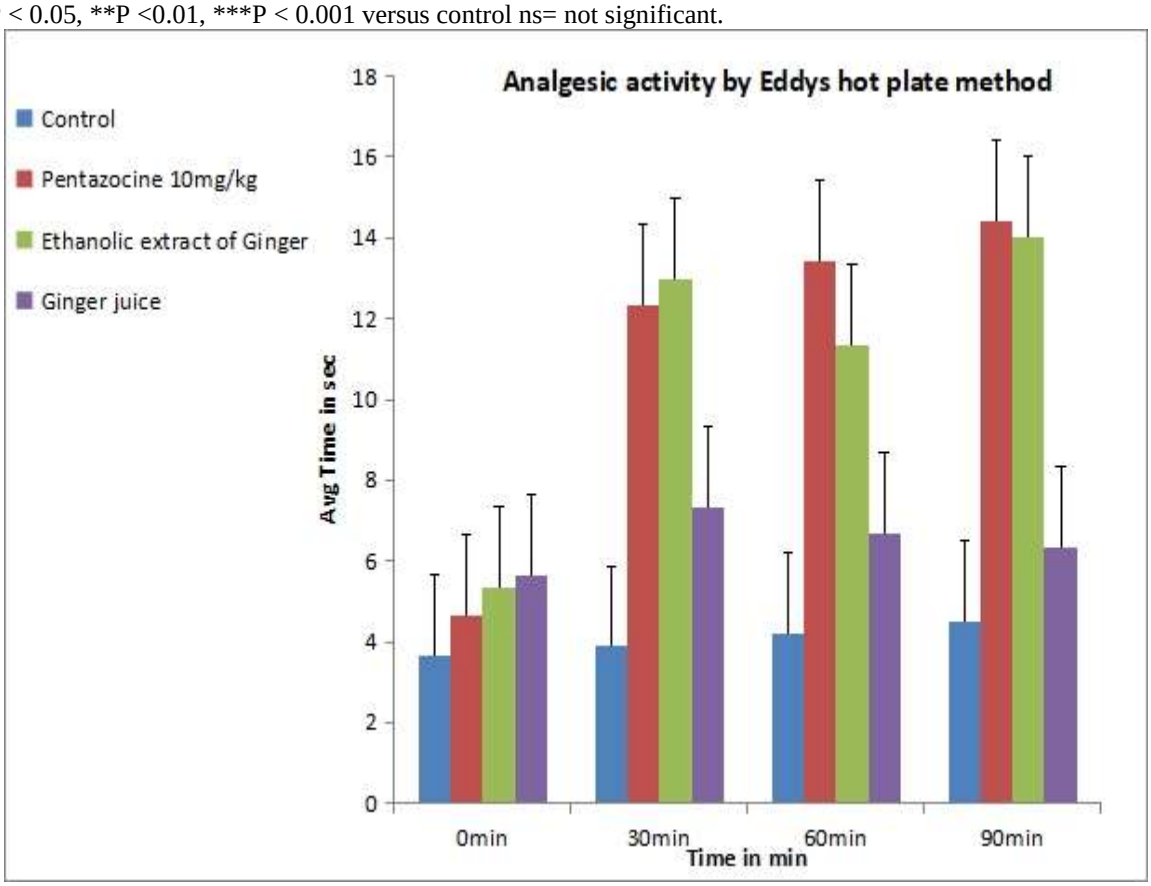

Figure 5.2.1.1 Results of eddy's hotplate method

Data was expressed as mean ± S.D., n=6
*****P < 0.05, **P <0.01, ***P < 0.001 versus control ns= not significant.

s. no.	**groups**	**Response**			
		0 min	30 min	60 min	90 min
1.	Group 1(control)	3.6±0.94	3.6±0.73	3.5±0.65	3.7±0.61
2.	Group 2(diclofenac sodium – standard)	4±0.81	4.9±0.85	4.5±1.14	10.3±1.69

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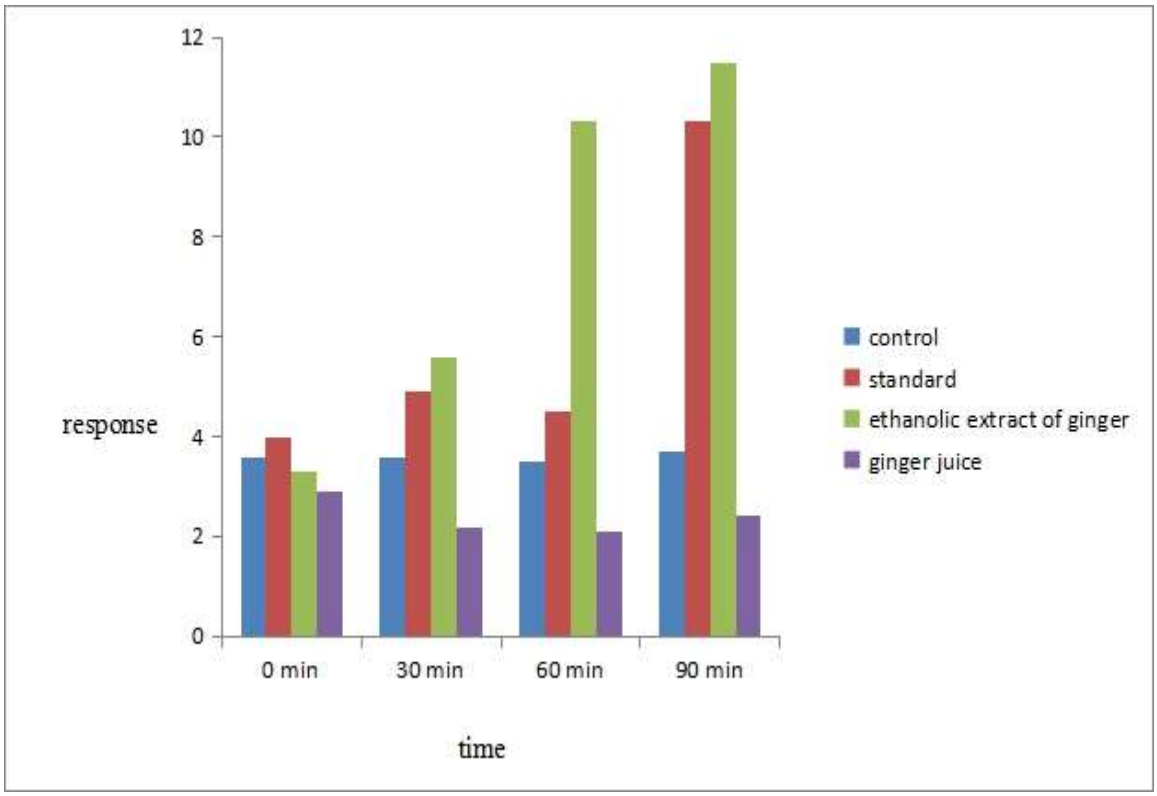
3.	Group 3(ethanolic extract of ginger)	3.3±0.47	5.6±1.69	10.3±1.73	11.5±0.65
4.	Group 4(ginger juice)	2.9±0.14	2.16±0.23	2.1±0.14	2.4±0.42

Table 5.2.2 Results of tail flick method



S.No	Groups	Treatment	Induction time(min)	No. of Writhings	%Effectivity
1	Group-I	Control	2.5±0.5	74±12	100%
2	Group-II	Diclofenac sodium 9mg/kg	5±0	6±1	8.1%
3	Group-III	Ethanolic extract of ginger 50mg/kg	3.5±1.5	7.5±0.5	10%
4	Group-IV	Ginger juice 50mg/kg	2.5±2.5	70.5±9.5	95.2%

Table 5.2.3 Results of Acetic acid induced writhings in mice.

Data was expressed as mean ± S.D., n=6
*P < 0.05, **P < 0.01, ***P < 0.001 versus control ns = not significant.

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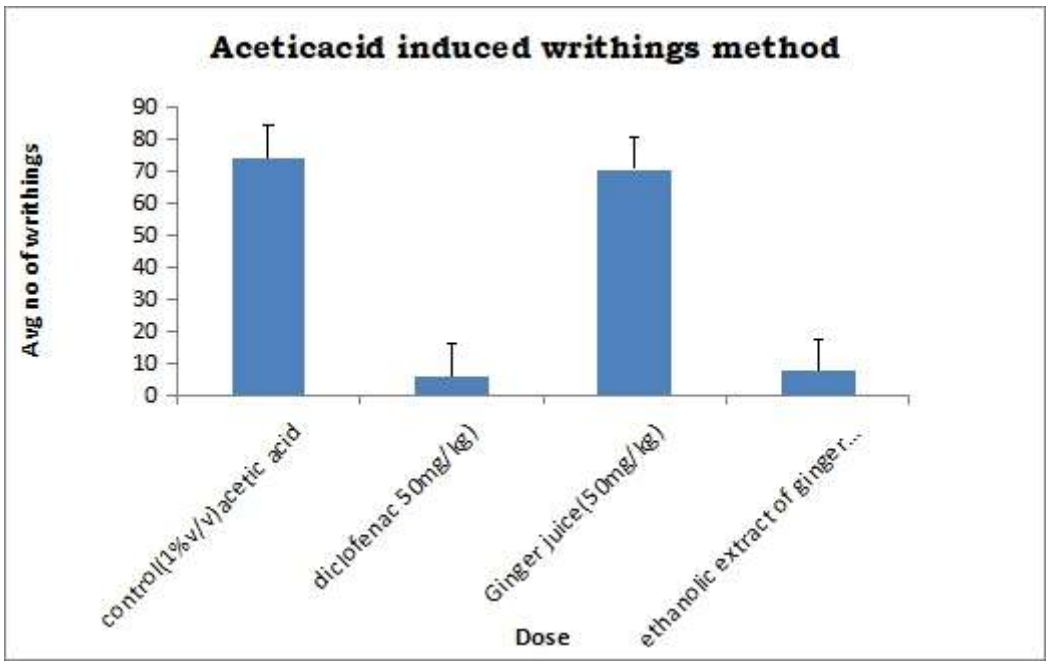

Figure 5.2.3.1 Results of acetic acid induced writhing method

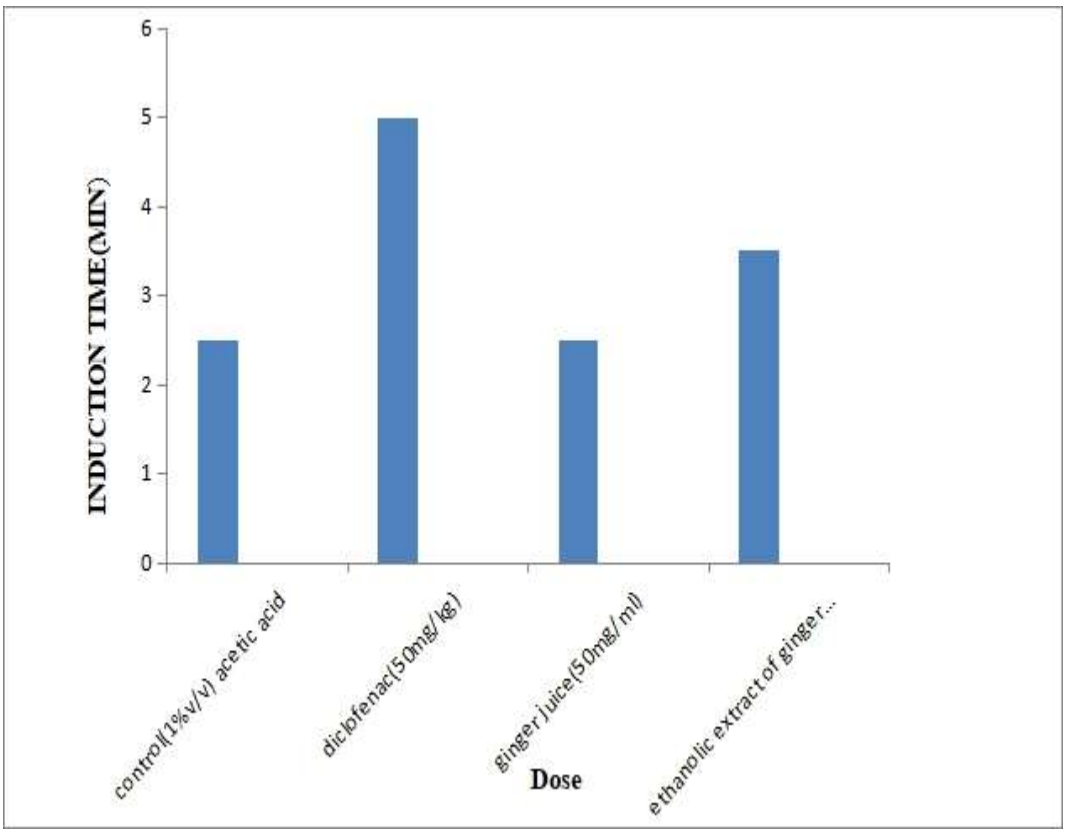

Figure 5.2.3.2 Results of acetic acid induced writhing method

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ANXIOLYTIC ACTIVITY

S.No	Groups	Treatment	No of entries in open arm			
			0min	30min	60min	90min
1	Group-I	Control	50±2.64	53.6±3.51	54.3±3.05**	54.3±2.08*
2	Group-II	Diazepam	50±2.64	71±5.12*	82±10.1^{ns}	95±28.5*
3	Group-III	Ethanolic extract of ginger 50mg/kg	54.4±5.46	30±3.1***	32±3.5***	34±5.1***
4	Group-IV	Ginger juice 50mg/kg	56.6±2.08	25±2.1***	30±3.09***	35±19.4***

Table 5.2.4 Results of anxiolytic activity by elevated plus maze method

Data was expressed as mean ± S.D., n=6
*P < 0.05, **P < 0.01, ***P < 0.001 versus control ns= not significant.

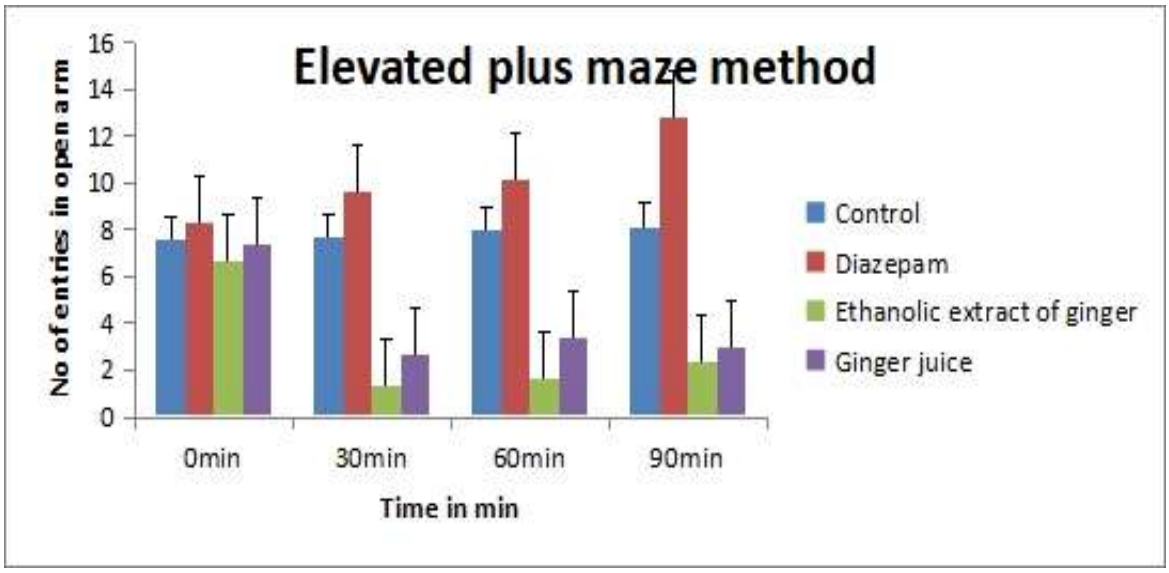

Figure 5.2.4.1 Results of elevated plus maze method

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SKELETAL MUSCLE RELAXANT ACTIVITY

S.No	Groups	Treatment	Fall off time			
			0min	30min	60min	90min
1	Group-I	Control	7.55±0.57	7.66±1.52**	7.99±1.57*	8.11±0.68**
2	Group-II	Diazepam	8.33±0.57	9.66±1.54***	10.1±0.57^{ns}	12.8±1.58**
3	Group-III	Ethanolic extract of ginger 50mg/kg	6.66±0.57	1.33±0.57**	1.66±0.57***	2.33±1.15**
4	Group-IV	Ginger juice 50mg/kg	7.33±1.52	2.66±1.52***	3.33±1.15**	3±1^{ns}

Table 5.4 Results of Skeletal muscle relaxant activity by Rotarod method

Data was expressed as mean ± S.D., n=6
*P < 0.05, **P < 0.01, *P < 0.001 versus control ns= not significant.

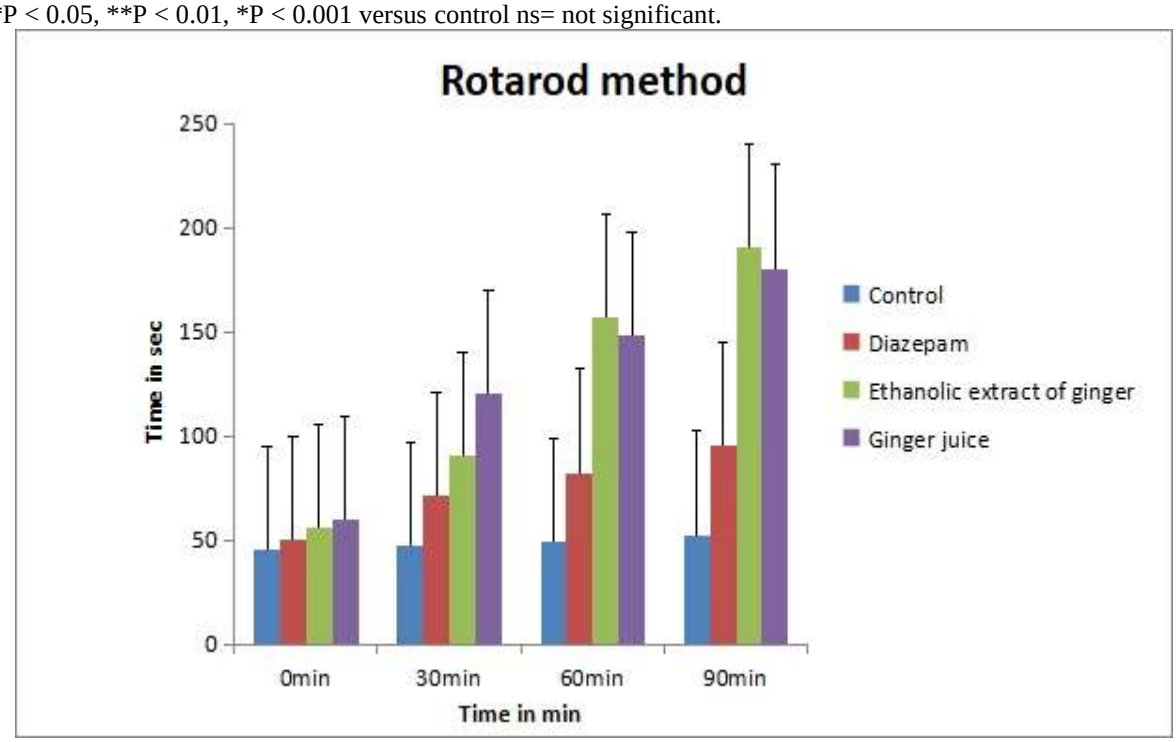

Figure 5.4.1 Results of rotarod method

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ANTI-MITOTIC ACTIVITY

Groups	Treatment	Weight of seeds in grams	**%** Inhibition
Group 1	Distilled water	0.6	0%
Group 2	Methotrexate	0.6	100%
Group 3	Ginger juice	0.5	60%
Group 4	Ethanolic extract of ginger	0.6	80%

Table 5.5 Results of Anti-mitotic activity by seed germination assay

VI. DISCUSSION

The phytochemical analysis of ginger extracts revealed the presence of alkaloids, carbohydrates, phenolic compounds, gum and mucilage, flavonoids, volatile oil whereas ethanolic extract of ginger showed the presence of terpenoids but not ginger juice.

Analgesic activity by using eddy's hot plate method when performed, the pentazocin and ethanolic extract of ginger groups have shown analgesic activity whereas no analgesic activity has shown by ginger juice. The activity shown by the ethanolic extract of ginger is less when compared to pentazocin treated group when it comes to acetic acid induced writhing test, diclofenac sodium and ethanolic extract of ginger groups have shown analgesic activity whereas no analgesic activity has shown by ginger juice. The induction time of ethanolic extract of ginger is less when compared to diclofenac sodium treated group. Significant increase in the reaction time for eddy's hot plate indicated the analgesic effect by ethanolic extract of ginger and also elucidates the involvement of central mechanism in analgesic action. Analgesic effect mediated through central mechanism indicates the involvement of endogenous opioid peptides and biogenic amines like 5HT. The terpenoids (cineole) were reported to have analgesic activity by inhibiting the production of cytokines(TNF- alpha, IL 1-beta), leukotriene B_4, Prostaglandin E_2.

Anxiolytic activity by using elevated plus maze method when performed, diazepam has shown anxiolytic activity whereas ginger juice and ethanolic extract didn't shown any anxiolytic activity.They didn't shown any anxiolytic activity because of the absence of cannabinoids as it's chemical constituents.

Skeletal muscle relaxant activity by using rota rod method when performed diazepam, ginger juice and ethanolic extract of ginger groups have shown muscle relaxant activity, but ginger juice and ethanolic extract of ginger have shown increased muscle relaxant activity when compared to diazepam treated group.The muscle relaxant activity shown by ginger juice and ethanolic extract of ginger is may be due to the presence of alkaloids, flavonoids, and terpenoids and due to the agonistic effect on GABA/benzodiazepine receptor complex.

Anti-mitotic activity by using seed germination assay when performed methotrexate, ethanolic extract of ginger and ginger juice shown anti-mitotic activity whereas control group i.e., distilled water group didn't show any anti-mitotic activity.The presence of sesquiterpenes in ginger were reported to have anti-mitotic activity by arresting the M phase of cell cycle.

VII. CONCLUSION

Chemical constituents like alkaloids, carbohydrates, phenolic compounds, gum and mucilage, flavonoids, volatile oil have been identified in both the extracts of ginger by performing phytochemical screening studies whereas ethanolic extract of ginger revealed the presence of terpenoids but not ginger juice. Ginger juice possess good muscle relaxant activity and also anti-mitotic activity. Ethanolic extract of ginger possess good analgesic, muscle relaxant and also anti-mitotic activity. Both ginger juice and ethanolic extract of ginger didn't show any anxiolytic activity.

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