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Research Article

ANXIOLYTIC AND ANALGESIC EFFECT OF SEEDS OF *CORIANDRUM SATIVUM* LINN

A. R. Pathan*, K.A. Kothawade and Mohd. Nadeem Logade

Singhania University, Jhunjhunu (Dt.), Pacheri-Bari, Rajasthan, India.

***Corresponding Author: aslamkhan_14@yahoo.com**

ABSTRACT

The clinical applications of benzodiazepines as anxiolytics are limited by their unwanted side effects. Therefore, the development of new pharmacological agents is well justified. Among medicinal plants, Coriandrum *sativum L*. has been recommended for relief of anxiety and insomnia in Iranian folk medicine. Nevertheless, no pharmacological studies have thus far evaluated its effects on central nervous system. Therefore, the aim of this study was to examine if the aqueous extract of *Coriandrum sativum* seed has anxiolytic effect in mice. Additionally, its analgesic effect was evaluated. The anxiolytic effect of aqueous extract (50, 100, 200 mg/kg, i.p.) was examined in male albino mice using elevated plus- maze as an animal model of anxiety. The effects of the extract on analgesic activity were assessed using Hot plate method. In the elevated plus-maze, aqueous extract at 200 mg/kg showed an anxiolytic effect by increasing the time spent on open arms and the percentage of open arm entries, compared to control group. Aqueous extract at 50, 100 and 200 mg/kg significantly produce analgesic activity compared to control group. These results suggest that the aqueous extract of *Coriandrum sativum* seed has anxiolytic effect and may have potential analgesic effects.

Keywords: *Coriandrum sativum*, Anxiolytic, Analgesic activity, Hot Plate Method.

INTRODUCTION

Anxiety disorders in a modern society have a relatively high prevalence and command considerable financial resources. Currently, the most widely prescribed medications for anxiety disorders are the benzodiazepines. However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, potentiation of other central depressant drugs and dependence liability. Therefore, the development of new medications possessing anxiolytic effect without the complications of benzodiazepines would be of great importance in the treatment of anxiety-related disorders. Medicinal plants are a good source to find new remedies for these disorders [2,3].

Anxiety

The primary use of sedative–hypnotic and anxiolytic drugs is to encourage calmness (anxiolytics or sedatives) or to produce sleep (sedative–hypnotics). All people are subject to states of emotional tension and uneasiness. For otherwise healthy individuals, these occasions are usually sufficiently mild and short that pharmacological intervention is unnecessary.

However, at times the symptoms of anxiety become quite discomforting and can interfere with a person's ability to function effectively[1].

Anxiety almost invariably accompanies many medical and surgical conditions, and it is often a symptom of psychiatric illness. When the symptoms become intolerable or interfere with the treatment of the underlying disease and if counseling is not sufficient, drug treatment can be considered as a means of helping patients cope with their anxiety[5,6].

All central nervous system depressants have some ability to relieve anxiety. However, most of these drugs relieve symptoms of anxiety only at doses that produce noticeable sedation. Drugs used to produce sedation and relieve anxiety are consistently among the most commonly prescribed drugs. Whether they are prescribed too frequently remains a matter of controversy. Insomnia includes a wide variety of sleep disturbances, such as difficulty in falling asleep, early or frequent awakenings, and remaining unrefreshed after sleep. Use of sedative–hypnotic drugs is one approach to the therapy of insomnia. Other measures include advice to avoid stimulants before retiring, maintenance of a proper diet, initiation of an exercise program, and avoidance of stressful or anxiety-provoking situations. Most anxiolytic and sedative–hypnotic drugs produce dose-dependent depression of central nervous system function. The ideal anxiolytic drug should calm the patient without causing too much daytime sedation and drowsiness and without producing physical or psychological dependence. Similarly, the ideal hypnotic drug should allow the patient to fall asleep quickly and should maintain sleep of sufficient quality and duration so that the patient awakes refreshed without a drug hangover. Also, both types of drugs should have very low toxicity and should not interact with other medications in such a way as to produce unwanted or dangerous effects[1,4].

BENZODIAZEPINES

The benzodiazepines constitute the most commonly used group of anxiolytics and sedative–hypnotics. Since the first member of this group, chlordiazepoxide, was introduced, many congeners have been marketed. Most of these drugs possess anxiolytic, sedative–hypnotic, and anticonvulsant properties. Thus, the clinical indications for specific benzodiazepines are not absolute and their uses overlaps considerably[12].

Mechanism of Action

The benzodiazepines bind with high affinity to specific macromolecules within the central nervous system. These benzodiazepine-binding sites (receptors) are closely associated with the receptors for gamma-aminobutyric acid (GABA), which is the major inhibitory neurotransmitter in the mammalian brain. Benzodiazepines potentiate GABAergic neurotransmission in essentially all areas of the central nervous system. This enhancement is thought to occur indirectly at the postsynaptic $GABA_A$ receptor complex.

The functional significance of this drug–receptor interaction is that the receptor complex regulates the entrance of chloride into the postsynaptic cells. The increase in chloride conductance mediated by GABA is intensified by the benzodiazepines. This facilitation of GABA-induced chloride conductance results in greater hyperpolarization of these cells and therefore leads to diminished synaptic transmission.

Another chemical class of sedative–hypnotic drugs, the barbiturates, also binds to receptors associated with the GABA–chloride ionophore, but these drugs appear to prolong rather than intensify GABA's effects. In addition to the clinically useful benzodiazepines, which act as agonists at the benzodiazepine receptor, at least two other types of ligands also interact with this binding site. These are the benzodiazepine receptor antagonists and the inverse agonists. For example, flumazenil (Romazicon) is a receptor antagonist that selectively blocks the effects of other benzodiazepines at their binding sites; it has clinical application in the treatment of benzodiazepine overdose and in the reversal of benzodiazepine-induced sedation. The inverse agonists are compounds that interact with benzodiazepine receptors and decrease, rather than increase, GABA mediated changes. They also can antagonize the

effects of benzodiazepine agonists and when administered alone, can be anxiogenic and proconvulsant[12].

Pharmacological Actions

Although it is widely claimed that the benzodiazepine drugs have a specific calming or anxiolytic effect, their most prominent and easily quantifiable action is central nervous system depression. In very low therapeutic doses, this depression manifests as relief of anxiety that is often accompanied by a feeling of sluggishness or drowsiness. As the dose is increased, the degree of depression is intensified such that muscle relaxation, hypnosis and a more intense central nervous system depression occur. This depression is related to the ability of these drugs to facilitate the inhibitory actions of GABA. A significant advantage of the benzodiazepines over other central nervous system depressants (e.g., the barbiturates) is that they possess a much greater separation between the dose that produces sleep and the dose that produces death. This increased margin of safety has been one of the major reasons benzodiazepines have largely replaced the barbiturates and other types of sedative–hypnotics in the treatment of anxiety and insomnia. In addition, benzodiazepine administration is associated with few side effects[12].

Clinical Uses

Anxiety

Anxiety disorders are among the most common forms of psychiatric illness. Anxiety often accompanies other psychiatric disease and such medical illnesses as angina pectoris, gastrointestinal disorders, and hypertension.

Anxiety that results from fear caused by an acute illness or a stressful event, such as a divorce or the loss of a loved one, is usually self-limiting and can be of relatively short duration. Other disorders that have anxiety as a component are not necessarily associated with a life event, and may persist for considerable periods, even throughout the individual's life. Both acute and chronic anxiety can be treated with benzodiazepines, although it is anticipated that for most anxiety disorders counseling will also play an important role. Benzodiazepines employed in the treatment of anxiety should be used in the lowest effective dose for the shortest duration so that they will provide maximum benefit to the patient while minimizing the potential for adverse reactions. For most types of anxiety, none of the benzodiazepines is therapeutically superior to any other. Choice of a particular agent is usually made on the basis of pharmacokinetic considerations.

A benzodiazepine with a long half-life should be considered if the anxiety is intense and sustained. A drug with a short half-life may have advantages when the anxiety is provoked by clearly defined circumstances and is likely to be of short duration[12].

PAIN

Pain has been described by the International Association for the Study of Pain as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." Although pain is a reaction of the body to harmful stimuli and is therefore a protective early warning system, the sensation of pain in postoperative patients, cancer patients, and other chronic pain patients has little positive effect. The stress response to pain can alter the healing process by evoking massive sympathetic discharge that in turn alters blood flow, tissue perfusion, and immune function. In addition, in certain painful conditions the patient has reduced respiratory function. Hence, the term pain, derived from the Latin poena for punishment, reflects the deleterious effects that can be inflicted upon the body. Since millions of Americans suffer from some form of pain each year, resulting in the expenditure of billions of dollars for various treatment modalities, pain and its underlying causes are a major public health problem[12].

Pain is warning signal that helps to protect the body from tissue damage. Potentially damaging stimuli activate and sensitize certain primary afferent nerve cells. Clinically, the sensation of pain elicits varying degrees of suffering and depression depending on its duration and patient's psychosocial environment[7].

Types of pain

Acute pain is defined as short-term but extreme pain that comes on quickly but last only for a brief period of time. Acute pain is centralized in one area before becoming somewhat spread out. This type of pain responds well to medications[8].

Chronic pain was originally defined as pain that has lasted 6 months or longer. It is now defined as pain that persists longer than the normal course of time associated with a particular type of injury. It is often more difficult to treat than acute pain. The experience of physiological pain can be grouped according to the source and related nociceptor. (pain detecting neurons)[12].

Somatic pain originates from ligaments, tendons, bones, blood vessels and even nerves themselves. It is detected with somatic nociceptors. The scarcity of pain receptors in these areas produces a dull, poorly-localized pain of longer duration than cutaneous pain; examples include sprains and broken bones[8].

Visceral pain The density of visceral nociceptors is <1% in comparison with somatic afferents and the cortical mapping of visceral afferents is also less concentrated[8].

Cutaneous pain is caused by injury to the skin or superficial tissues. Cutaneous nociceptors terminate just below the skin and due to the high concentration of nerve endings, produce a well-defined, localized pain of short duration[9].

Neuropathic pain or "neuralgia" can occur as a result of injury or disease to the nerve tissue itself. This can disrupt the ability of the sensory nerves to transmit correct information to the thalamus and hence the brain interprets painful stimuli even though there is no obvious or known physiologic cause for the pain[10].

Phantom limb pain is the sensation of pain from a limb that has been lost or from which a person no longer receives physical signals. It is an experience almost universally reported by amputees and quadriplegics[11].

Pathways of Pain

Nociception is conveyed from the periphery to the brain by an adaptable and dynamic pathway. The pathway is transmitted and modulated at three levels: the peripheral nociceptor, the spinal (dorsal horn of the cord) and the supraspinal (brain).

Peripheral activation

Most pain originates after tissue damage. The release of inflammatory mediators from tissues, immune cells and sympathetic and sensory afferent nerve fibers results in an 'inflammatory soup' bathing the nociceptors. These chemicals can directly activate or sensitize the high-threshold nociceptors to activation by low intensity stimuli[11,12].

Spinal level

The dorsal horn of the spinal cord is the site where complex interconnections occur between excitatory and inhibitory interneuron's and the descending inhibitory tracts from the brain. The second-order neurons are of two types: nociceptive specific (in laminae II and III), which respond selectively to high threshold nociception and wide dynamic range or convergent neurons (in laminae V and VI), which respond to a range of inputs [11].

Neurotransmitters at the dorsal horn

Many neurotransmitters play a role in nociceptive transmission in the dorsal horn. The transmitters fall into two groups; excitatory amino acids and Neuropeptides. They activate the ionotropic α-amino-3-hydoxy-5-methyl-4-isoazolepropionate and neurokinin-1 receptors, respectively, to produce ionic depolarization of the cell. Analgesic drugs targeted at the spinal cord level include local anaesthetics (produce non-specific conduction blockade) and opioids (act on opioid receptors)[11].

Supraspinal level

The supraspinal function in nociception is beginning to be explored with the advent of non-invasive imaging, such as functional MRI and PET. The perception of pain is associated with changes in activity of the thalamus, primary and secondary cortex and particularly the anterior cingulated cortex.

The inhibitory pathway then descends the spinal cord via the dorsal columns and terminates at the dorsal horn where neurotransmitters (noradrenaline, 5-hydroxytryptamine) and the endogenous opioids are released to provide

antinociception. The three receptors (mu, delta, kappa) play a role in the ascending pathways but the mu and delta receptors are mainly responsible in the descending component[8].

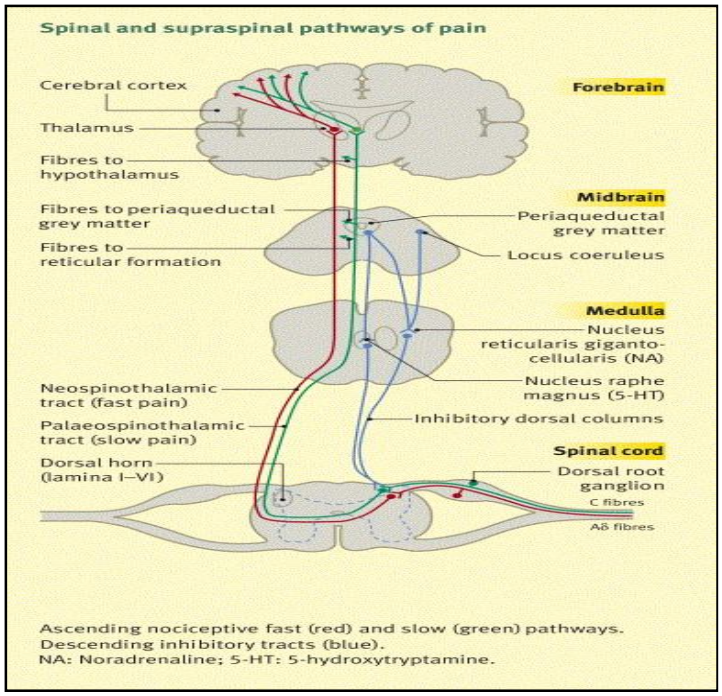

Fig. 1: Spinal and supraspinal pathways of pain[8]

Synthesis and release of noradrenaline and 5-hydroxytryptamine are increased by opioids and they in turn enhance the action of opioids. This may be the mechanism by which the antidepressants and tramadol work as analgesics[8].

Mediators of inflammation and Pain

A mediator of inflammation is defined as any messenger that acts on blood vessels, inflammatory cells or other cells to contribute to an inflammatory response. They originate from plasma of cells, when in plasma are found in inactive stage and must be activated. Microbial products or host proteins trigger production of active mediators. One mediator can stimulate the release of other mediators by target cells (provide mechanism of amplification). Most of the mediators are short lived[13].

Table 1: Mediators in Inflammation and Pain[13]

Vasodilation	Increased Vascular Permeability	Chemotaxis Leukocyte Activation	Fever	Pain	Tissue Damage
PG, NO	Vasoactive amines, C3aand C5a (through liberating Vasoactive amines from cells), Bradykinin, LTC4, D4, E4, PAF	C5a, LTB4 Chemokines (e.g. IL-8)	IL-1, IL-6, TNF-α, PG	PG Bradykinin	Neutrophil & Macrophage Products Lysosomal Enzymes Oxygen Metabolite, NO

Table 2: Some of the mediators in acute inflammation and their effects

Mediators	Vasodilatation	Vascular Permeability	Chemotaxis	Pain
Histamine	++	↑↑↑	-	-
Serotonin	+/-	↑	-	-
Bradykinin	+++	↑	-	+++
Prostaglandin	+++	↑	+++	+
Leukotrienes	-	↑↑↑	+++	-

EXPERIMENTAL ANIMALS

Male albino mice weighing 22–28 g were used. animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Institutional Animal Ethics Committee approved the experimental protocol 2011/SU/6/CPCSEA. Albino mice were used in this thesis was obtained from the Bioneeds Animal House Pune (Maharashtra). The animals were given standard diet supplied by Pranav Agro Industries Ltd. Sangli. The animals had free access of standard diet and water and housed in a spacious cage for one week. The composition of the diet are protein 10%, Arachis oil 4%, Fibers 1%, Calcium 1%, Vitamin A 1000IU/gm Vit D 500IU/gm.

Mice were housed in cages of 5 at 22 ± 1◦C in a 12- h light/dark cycle. Tap water and food pellets were available as libitum. Groups of 6–11 mice were randomly assigned to different treatment groups and were tested in a counter balancing order. Animals were naive to experiment conditions. All experiments were carried out in a quiet room under dim red light between 9:00 a.m. and 2:00 p.m. After procuring the animals kept under standard husbandry conditions as follows:

Room temperature –26±2⁰
Relative humidity –45 - 55%
12 hours light/dark cycle

Collection of Crude Drug

Dried seeds of coriander were purchased from a commercial source in North Maharashtra (Nashik). The identity of the seed was confirmed by the Botanical Survey of India, Koregaon Park, Pune (Maharashtra). A voucher specimen was kept in our laboratory for future reference.

Preparation of Aqueous Extract

Dried coriander seeds were homogenized to a fine powder. Hundred grams of powdered coriander was infused in 500 ml cold ethanol for 24 h, brought to the boil, then removed from the heat source and allowed to infuse for 15 min. The extract was filtered, may concentrated over the water bath and brought to dryness under vacuum. The yield of the extract was 7.9% (w/w)[15,16].

PHYTOCHEMICAL INVESTIGATION OF EXTRACT

The extracts of *Coriandrum sativum* Linn. was subjected to qualitative analysis for the various phytoconstituents. Tests for common phytochemicals were carried out by standard methods [17,18]

List of Materials Used

- Alcohol (Hi-Media, Mumbai)
- Diazepam (Lupin , Mumbai)
- Morphine Sulphate (Lupin, Mumbai)
- CMC (Hi-media, Mumbai)

Acute oral Toxicity Studies[19]

Acute oral toxicity - Acute toxic class method

The acute oral toxicity was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

It is the principle that based on a stepwise procedure with the use of a minimum number of animals per step to obtain sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined

doses. The substance is tested using a stepwise procedure, each step using three animals of either sex. Absence or presence of compound related mortality of the animals dosed at the step will determine the next step of

- No further testing is required
- Dosing of three additional animals with the same dose
- Dosing of 3 animals at the *next* higher or the next lower dose level

The method enables judgment with respect to classifying the test substance to one of the series of toxicity classics defined by fixed LD_{50} cut off values.

Description of the methods

1) Selection of animal species

Healthy young female mice weighing 22-28 gm were used for acute toxicity study to determine LD_{50} of extract. Each group contains 3 animals.

2) Housing and feeding condition

The temperature in the experimental room was around 25°c. Lighting was natural sequence being 12 hours dark, 12 hours light. The conventional laboratory diet was fed with adequate supply of drinking water.

3) Preparation of Animals

The animals were randomly selected, marked to permit individual identification and kept in polypropylene cages for one week prior to dosing to allow acclimatization of them to laboratory conditions.

4) Preparation of doses

The drugs were prepared as a suspension by triturating with water and 0.5% sodium CMC.

5) Administration of doses

The test substances were administered in a single dose by using a mice oral feeding needle. Prior to dosing, animals were kept for 12 hours of fasting. Then animals were weighed and test substance was administered. After the administration of dose, food was withheld for a further 3-4 hours.

6) Number of animals and dose levels

In each step three animals were used in each group. Study was begin at 50 mg/kg body weight and continued up to 5000 mg/kg body weight. The procedure of dose selection and finalizing LD_{50} cut off values is as below.

Table 3: Acute oral toxicity study

S. No.	Name of extract	LD_{50} cut off mg/kg body weight	Vehicle
1.	AECS	5000	Sodium CMC

$1/10^{th}$ of this lethal dose was taken as effective dose (therapeutic dose) for subsequent anti-inflammatory and analgesic activity.

Observations

Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours. In above case death was observed within first 24 hours. Additional observations like changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system and lomatomotor activity and behavior pattern. Attention was also given to observation of tremors and convulsions.

So we had selected three doses, i.e 50 mg/kg, 100 mg/kg and 200 mg/kg body weight as low dose, medium dose and high dose respectively for the present anxiolytic and analgesic activity.

1. ANXIOLYTIC EFFECT

- **Methods-** Elevated Plus Maze Test [14,15].
- **Equipment**- Elevated Plus Maze apparatus.

Principle

Elevated plus-maze is the most simple apparatus to study anxiolytic response of almost all type of anxiolytic agents. Exposure of animals to novel maze alley evokes an approach-avoidance conflict which is stronger in open arm as compared to enclosed arm. Rodents (rats & mice) have aversion for high & open space and prefer enclosed arm and, therefore, spend greater amount of time in enclosed arm. When animals enter open arm, they freeze, become immobile, defecate and show fear-like movements. The plasma cortisol level is also reported to be increased, as a true reflection of anxiety. Major advantages of this test procedure

are- (a) it is simple, fast and less time consuming, (b) no prior training or noxious stimuli (sound o r light) is required, and (c) it is predictable and reliable procedur for studying anxiety response as well as anxiolytic action of drug [20].

Animals -- Albino mice
Weight -- 22 to 28 gms.
Sex -- either sex

Sample Preparation

1. Control- Saline soiution I p.

2. Std- Diazepam (3mg/kg ip) was prepared as stock solution containing 0.3 mg/ml of the drug & was injected 1ml/100 g of body weight of the mouse. Diazepam was suspended in 1% w/v gum acacia or corboxymethylcellulose.

3. Test- Coriander extract 50, 100 & 200 mg/kg.

Animal groups

Each group were contain 5 animals
Group-I: was received Control (Saline) solution.
Group-II: was received Std (Diazepam) drug.
Group-III: was received Test-1 (Coriander extract-50mg/kg) drug.
Group- IV: was received Test-2 (Coriander extract-100mg/kg) drug.
Group- V: was received Test-3 (Coriander extract-200mg/kg) drug.

Procedure

1. Animals was weighed, numbered & divided them into six groups each containing of 5 mice. One group is used as control (saline), second for drug (Diazepam) std. treatment & third, fourth & fifth group for coriander extract treatment (Test).
2. Animals were placed individually in the centre of the maze, head facing towards open arm & stop watch was started & following parameters was noted for five minutes
a) First preference of mouse to open or enclosed arm.
b) Number of entries in open & enclosed arms.
(an arm entry defined as the entry of four paws into the arm.)
c) Average time each animal spends in each arm
(Average time = total duration in the arm / number of entries).
3.Saline & Diazepam were injected to the control & std . groups respectively. After 30 minute animals was placed individually in the centre of the maze & all parameters were noted as under step 2.
4.Coriander extract were injected to the test group. After 30 minute animals was placed individually in the centre of the maze & all parameters were noted as under step 2.
5.Finally we compared the preference of the animals to open or enclosed arm, average time spend in open arm & number of entries in open arm in each group.

2. Analgesic Effect

Method- Hot Plate Method[14].
Equipment- Eddy's hot plate.

Principle

Analgesia is defined as state of reduced awareness to pain, and analgesics are substances which decrease pain sensation (pain-killers) by increasing threshold to painful stimuli. The commonly used analgesics are aspirin, paracetamol (non-narcotic type) and morphine (narcotic type). Painful reaction in experimental animals can be produced by applying noxious (unpleasant) stimuli such as (a) thermal (radiant heat as a source of pain), (b) chemical (irritant such as acetic acid and bradykinin) (c) physical pressure (tail compression). In the laboratory, commonly used procedures are tail –flick (tail withdrawal from radiant heat) method using analgesiometer, hot plate (jumping from hot plate at 55 C) method and acetic acid- induced writhing [20].

Animals -- Albino mice
Weight -- 22 to 28 gms.
Sex -- either sex.

Sample Preparation

1. Control- Saline soiution i. p.

2.Std.- Morphine sulphate (5mg/kg ip) was Prapared as stock solution containing 0.5 mg/ml of the drug & injected 1ml/100 g of body weight of the mouse.

3. Test- Coriander extract 50, 100 & 200 mg/kg.

Animal groups

Each group was contain 5 animals
Group-I: was received Control (Saline) solution.

Group-II: was received Std (Morphine) drug.
Group-III: was received Test-1 (Coriander extract-50mg/kg) drug.
Group- IV: was received Test-2 (Coriander extract-100mg/kg) drug.
Group- V: was received Test-3 (Coriander extract-200mg/kg) drug.

Procedure

1. Animals were weighed, numbered & divided them into six groups each containing of 5 mice. One group was used as control (saline), second for drug (Morphine sulphate) std. treatment & third, fourth & fifth group for coriander extract treatment (Test).

2.Basal reaction –time were noted by observing hind paw licking or jump response (whichever appears first) in animal when placed on the hot plate maintained at constant temp (55C). normally animals show such response in 6 -8 sec. a cut off period of 15 sec was observed to avoid damage to the paws.

3.Saline, Morphine & coriander extract were injected as per animals groups & reaction time of animals was noted on the hot plate at 15, 30, 60, & 120 min after the drug administration.

4. Percent increase in reaction time (as index of analgesia) at each time interval was calculated.

PIP was calculated using following formula

$$\text{PIP} = \frac{Tt - Tc}{Tc} \times 100$$

Where ,

Tc = Time required to lick a paw in control animal

Tt = Time required to lick a paw in treated animal

STATISTICAL ANALYSIS

The statistical significance was assessed using one –way analysis of variance (ANOVA) followed by Dunnet comparison test. The values are expressed as mean ± SEM and $p<0.05$ was considered significant.

Anxiolytic Activity

Elevated plus-maze Method

Dunnett's *t*-test revealed that administration of diazepam (3 mg/kg) significantly increased the amount of time spent in the open arms ($P < 0.001$), compared to saline-treated group. The aqueous extract of coriander seeds at 200 mg/kg significantly increased the time spent in the open arms ($P< 0.001$) compared to saline-treated group (table 4). There were significant differences between the coriander extract and saline-treated groups for the number of open arm entries.

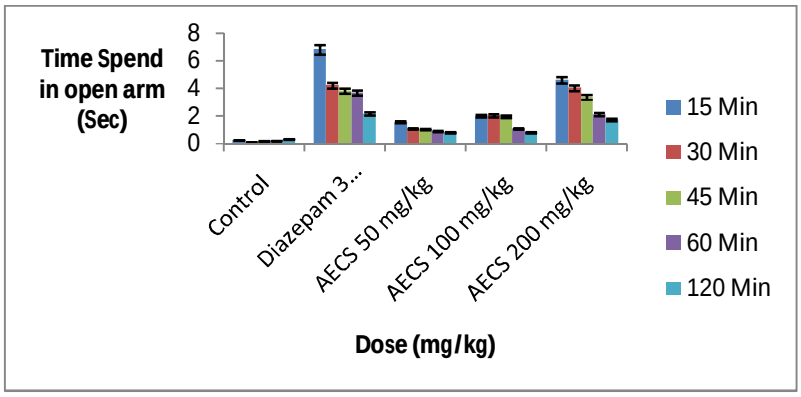

Fig.2: Anxiolytic Effect of alcoholic Extract of *Coriandrum sativum* L. on Elevated Plus Maze method in mice. (Time spend in open arm)

Table 4: Anxiolytic Effect of alcoholic Extract of *Coriandrum sativum* L. on Elevated Plus Maze method in mice. (time spend in open arm)

Time Interval (Min)	GROUPS [No. of Entries in open arm (Sec)]				
	Control	Diazepam (5mg/kg)	AECS 50(mg/kg)	AECS 100(mg/kg)	AECS 200(mg/kg)
15	0.25 ± 0.02	6.81 ± 0.27***	1.57 ± 0.11***	2.01 ± 0.24***	4.59 ± 0.20***
30	0.11 ± 0.05	4.2 ± 0.30***	1.08 ± 0.28***	2.03 ± 0.25***	4.03 ± 0.29***
45	0.18 ± 0.05	3.82 ± 0.26***	1.04 ± 0.20***	1.98 ± 0.17***	3.36 ± 0.24***
60	0.21 ± 0.05	3.68 ± 0.27***	0.91 ± 0.05*	1.08 ± 0.13***	2.12 ± 0.16***
120	0.32 ± 0.03	2.18 ± 0.39***	0.81 ± 0.07^{NS}	0.82 ± 0.04^{NS}	1.75 ± 0.23***

Values are expressed in Mean ± SEM
Statistical analysis was done by using one way ANOVA followed by Dunnet's t-test
*P < 0.05, *** P < 0.001, N = 6, NS= Not Significance

Analgesic Activity
Hot plate Method

AECS produced significant analgesic activity ($P < 0.001$) against Hot Plate method was in dose dependent manner. The Analgesic effects of the AECS by the hot plate method in mice were summarized in (Table 5). Pretreatment with the extract significantly increased reaction time in comparison to control. At a dose of 200 mg/kg of AECS exhibited analgesic effect and reaction time was 5.281 sec. while standard drug Morphine 5 mg/kg i.p. had reaction time of 6.282 sec.

At a dose of 200 mg/kg AECS shows more than 50% Pain Inhibition Percentage (PIP) as compared to control. (Figure-4)

Table 5: Effect of alcoholic Extract of *Coriandrum sativum* L. on Hot plate method in mice. (Reaction time)

Reaction Time (Sec)	GROUPS				
	Control	Morphine (5mg/kg)	AECS 50(mg/kg)	AECS 100(mg/kg)	AECS 200(mg/kg)
15	0.599 ± 0.005	6.102 ± 0.11***	0.915 ± 0.15**	1.920 ± 0.07***	5.281 ± 0.27***
30	0.601 ± 0.018	6.282 ± 0.27***	0.868 ± 0.05^{NS}	2.516 ± 0.18***	4.398 ± 0.29***
45	0.403 ± 0.014	5.208 ± 0.29***	0.715 ± 0.09^{NS}	1.952 ± 0.25***	3.881 ± 0.27***
60	0.418 ± 0.030	4.102 ± 0.31***	0.798 ± 0.12^{NS}	1.222 ± 0.25***	2.014 ± 0.26***
120	0.378 ± 0.021	2.011 ± 0.42***	0.594 ± 0.05^{NS}	0.650 ± 0.06^{NS}	0.812 ± 0.05^{NS}

Values are expressed in Mean ± SEM
Statistical analysis was done by using one way ANOVA followed by Dunnet's t-test
***P < 0.001, ** P < 0.01, N = 6, NS = Not Significance

Table 6: Effect of alcoholic Extract of *Coriandrum sativum* L. on Hot plate method in mice. (PIP)

Group	Pain Inhibition Percentage (PIP)				
	15 min	30 min	45 min	60 min	120 min
Control (CMC 1%)	-	-	-	-	-
AECS 50 mg/kg (i.p.)	52.75	44.42	77.41	90.90	57.14
AECS 100 mg/kg (i.p.)	220	318	384	192	71.95
AECS 200 mg/kg (i.p.)	781	631	863	381	114
Standard (Morphine 5 mg/kg i.p.)	918	945	1192	925	566

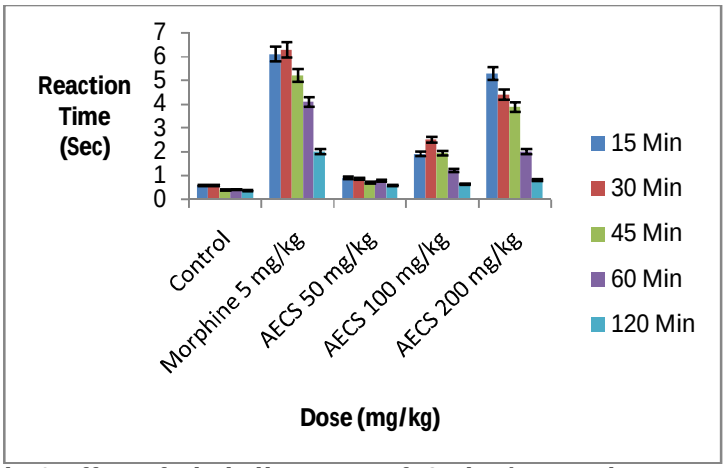

Fig. 3: Effect of alcoholic Extract of *Coriandrum sativum* L. on Hot plate method in mice. (Reaction time)

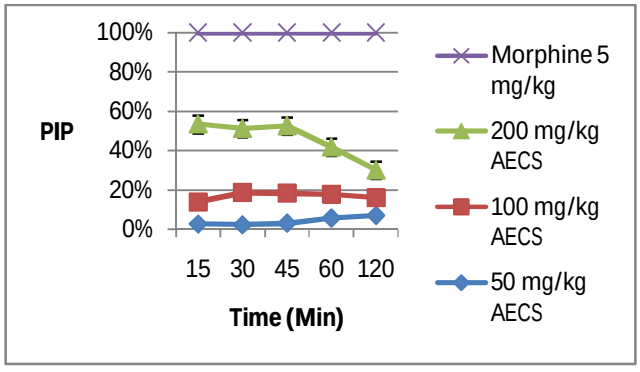

Fig. 4: Effect of alcoholic Extract of *Coriandrum sativum* L. on Hot plate method in mice. (Pain Inhibition Percentage)

DISCUSSION

The Present study reveals that seeds of Coriandrum sativum possesses both Anxiolytic and Central Analgesic activity.

The elevated plus- maze is currently one of the most widely used models of animal anxiety[5] and has been validated for use with both rats and mice Therefore , we chose this test to investigate the anxiolytic potential of the aqueous extract of coriander seed. The indices of anxiety in this test, percent of open arm entries and time spent in the open arm are sensitive to agents thought to act via the $GABA_A$ receptor complex, justifying the use of diazepam as a positive control in this study. In agreement with previously published reports, diazepam increased the percentage of open arm entries and the time spent in the open arms confirming its anxiolytic effects [3,21]. The aqueous extract of coriander seed had similar effects on these parameters. The effect of 200 mg/kg coriander on the elevated plus –maze test was almost equivalent to that of 0.3 mg/kg diazepam. These observations clearly indicate that coriander seed exerts an anxiolytic activity.

In the present study, the anxiolytic activity of the coriander extract occurred at a dose of 200 mg/kg in mice. Although we should be cautious in extrapolating the dose obtained from animal studies to human subjects, it may be suggested that the effective dose for a 75 kg adult man would be 7.5 g dry extract of coriander seed. This corresponds to an infusion of approximately 20 g of coriander seed in 100 ml water, considering the yield of the extract. This is in the range of the coriander doses empirically used in traditional medicine. However, the optimum therapeutic dose for human would require further studies, evaluating the effect of the extract in a clinical situation.

The results of hot- plate test showed that Coriander Extract provide significant protective effects on thermic pain stimuli. Such an effect is a characteristic of the central analgesic effect, like morphine, while peripheral analgesic is known to be inactive on this kind of painful stimuli.

Dunnett's *t*-test revealed that administration of diazepam (3 mg/kg) significantly increased the amount of time spent in the open arms ($P < 0.001$), compared to saline - treated group. The aqueous extract of coriander seed at 200 mg/kg significantly increased the time spent in the open arms ($P< 0.001$) compared to saline –treated group (Table 4). There were no significant differences between the coriander extract and saline –treated groups for the number of close arm entries.

AECS produced significant analgesic activity ($P < 0.001$) against Hot Plate method was in dose dependent manner. The Analgesic effects of the AECS by the hot plate method in mice were summarized in (Table 5). Pretreatment with the extract significantly increased reaction time in comparison to control. At a dose of 200 mg/kg of AECS exhibited analgesic effect and reaction time was 5.281 sec. while standard drug Morphine 5 mg/kg i.p. had reaction time of 6.282 sec. At a dose of 200 mg/kg AECS shows more than 50% Pain Inhibition Percentage (PIP) as compared to control.

CONCLUSION

1. The qualitative Phytochemical study reveals the presence of sterols, carbohydrates, proteins, terpenes, flavanoids and tannins.
2. The present study demonstrated that the alcoholic extract of seeds of *Coriandrum sativum* L. possess Anxiolytic and analgesic activity.
3. The Anxiolytic effect, percent of open arm entries and time spent in the open arm are sensitive to agents thought to act via the $GABA_A$ receptor complex.
4. The central analgesic action of the *Coriandrum sativum* was probably mediated through inhibition of central pain receptors.
5. The above effects of it may also be due to the presence of Sterols, tannins and flavanoids in the extract.

Scope for further study

Further their is need to isolate, characterize and screen the active principles from the different parts of *Coriandrum sativum* L. that are responsible for its Anxiolytic and analgesic activity. Also their is need to find out the exact mechanism by which the plant exerts above effects.active principles from the different parts of *Coriandrum sativum* L.
may be evaluated for following activities-

- ✓ Anti-convulsant activity

- ✓ Locomotor activity
- ✓ Muscle-Relaxant activity.

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