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Formulation and Evaluation of Novel Drug Delivery System of Passiflora Incarnata for Antianxiety Activity

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Abstract**—***At the present time, anxiety is among the most common mental illnesses. The main causes of anxiety are psychological stress, traumatic damage, stress brought on by various medical conditions, genetic factors, drug and alcohol abuse, and other mental health conditions. Anxiety can have a major impact on a person's day-to-day functioning. It can lead to decreased coordination, an irregular sleep schedule, difficulty concentrating at work, appetite loss, and an elevated risk of suicide, particularly in those who are depressed. Benzodiazepines (diazepam, lorazepam, oxazepam), azapirones (Busipirone, Gepirone), antidepressants such as SSRIs (Paeoxetine, Fluoxentine), and SNRIs (Duloxetine, Venlafaxine) are being used to treat anxiety. Anxiety disorders can also be treated with other medications, such as tricyclic antidepressants (Clomipramine, Imipramine), antihistaminics (Hydroxyzine), and MOIs (Tranylcypromine). Compared to pricey synthetic medications, herbs can be a highly effective preventive medicine and be of significant value when included in a health programme. Numerous allopathic formulations are employed, such as clonazepam, lorazepam, alprazolam, and diazepam, however they have major adverse effects such diarrhoea, fast heartbeat, vertigo, nausea, and psychosis. Benzodiazepines and antidepressants are frequently used in modern allopathic treatment to treat anxiety disorders. However, there are side effects including vertigo and depression linked to these medications. Studies indicate that extract from Passiflora Incarnata may reduce anxiety and associated diseases via modulating the GABA A receptor, as evidenced by the plant's aerial part's antianxiety activity. It has been noted that Passiflora Incarnata has better therapeutic activity and is safer and more effective. According to recent studies, Passiflora Incarnata has good sedative and anxiolytic effects on anxiety. Anxiety, sadness, and related mental disorders may arise as a result of elevated oxidation and inflammation, according to recent studies that suggests these conditions may impact specific brain regions. Our study leads us to conclude that the combination of an anxiolytic medication with an antioxidant and an anti-inflammatory agent may result in greater anxiolytic activity and synergistic activity*.

Keywords**—** *Anxiety, Passiflora incarnata L.*

I. INTRODUCTION

Anxiety:-

Anxiety is characterised by anxiousness, unease, and fear. You might start to perspire, have tension and restlessness, and blink your eyes quickly like a fire. It can be a typical reaction to stress. For example, you may have anxiety prior to an assessment, when faced with a sensitive issue at work, or prior to making a significant decision. It can support your management. Your anxiety might help you focus or give you an energy boost. However, fear is not fleeting and can even be tempting for those who suffer from anxiety disorders [1].

Anxiety Disorder:-

It's common to have anxiety before a test, a job interview, or a move. Although this kind of anxiousness is undesirable, it could inspire you to put in more effort and produce better work. Anxiety that is typical is a fleeting emotion that doesn't interfere with day-to-day activities. Should you have an anxiety ailment, you might always feel afraid. It's sometimes crippling and brutal. Anxiety of this kind may cause you to give up activities you enjoy. In extreme circumstances, it might prevent you from walking outside your house, using a lift, or crossing the street. But the nervousness will just intensify. if not covered.

The most prevalent emotional complaint is anxiety disorders, which can afflict people of any age. The American Psychiatric Association reports that women are diagnosed with anxiety complaints at a higher rate than men [2].

Several types of anxiety disorders exist [3]

"Agoraphobia, an anxiety disorder, induces intense fear of particular situations, leading some individuals to avoid leaving their homes altogether."

"Anxiety disorder stemming from a medical condition manifests symptoms of severe anxiety or panic triggered directly by a physical health issue."

"In general, anxiety disorders When challenged with ordinary or regular everyday events or circumstances, people with generalised anxiety disorder react with excessive concern and anxiety.

"*Panic disorder* entails recurrent episodes of sudden and intense anxiety and fear, known as panic attacks, which peak rapidly. During these episodes, individuals may experience sensations of impending doom, shortness of breath, chest pain, or a fast, irregular, or pounding heartbeat. Fear of

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experiencing these attacks again or avoiding situations where they've occurred may result from these panic attacks."

" *Selective mutism* occurs when kids fail to interact in certain circumstances like school, but can in others, such at home with their closest relatives."

"*Separation anxiety disorder* in children is marked by an anxiety level that surpasses typical developmental stages, specifically triggered by separation from parents or individuals assuming parental roles."

"Social anxiety disorder, also known as social phobia, entails intense anxiety, fear, and avoidance of social interactions stemming from worries about embarrassment, self-consciousness, and the fear of being negatively evaluated or judged by others."

"*Specific phobias*, is one experiences intense fear upon seeing a certain thing or circumstance, which makes one feel strongly inclined to avoid it."

"*The symptoms of intense anxiety or panic brought on by substance abuse, medicine usage, exposure to toxins, or withdrawal from substances are exhibited by substance-induced anxiety disorder*."

"*Anxiety or phobias* that do not exactly meet the criteria for any other recognised anxiety disorders, but are still significant enough to cause distress and disruption, are referred to as other specified anxiety disorder and unspecified anxiety disorder."

Anxiety Disorder Symptoms [4]

The primary symptom of anxiety disorders is excessive fear or worry, which can also manifest as difficulty breathing, sleeping, remaining still, or concentrating. Depending on the kind of anxiety condition, different symptoms apply.

The common symptoms include

- Panic, fear, and uneasiness
- Feelings of panic, doom, or danger
- Sleep problems
- Being unable to remain silent and composed
- Cold, sweaty, numb, or tingling hands or feet
- Shortness of breath
- Hyperventilation, or breathing more quickly and strongly than usual
- Heart palpitations
- Dry Mouth
- Nausea
- Tense muscles
- Dizziness
- Rumination is the inability to put an idea aside and returning to it.
- Inability to concentrate
- Intensely or compulsively avoiding locations or things that cause dread.

Risk Factor [5]

Researchers are investigating the combined influence of both genetic and environmental factors on the likelihood of developing an anxiety disorder. While the specific risk factors for each type of anxiety disorder may differ, some general risk factors include:

- Shyness or experiencing worry or nervousness in new situations during childhood
- Exposure to stressful or negative life events or environmental factors
- A family history of anxiety disorders or other internal illnesses
- Anxiety symptoms that can be triggered or worsened by:
- Certain physical health conditions, such as thyroid issues or heart arrhythmias
- Consumption of caffeine or other substances
- Obtaining a physical examination from a healthcare professional can help identify the cause of your symptoms and the best course of action if you think you may have an anxiety condition.

Causes [6]

There is still much to learn about the causes of anxiety disorders. However, life events such as traumatic experiences seem to trigger anxiety disorders in individuals who are already predisposed to anxiety. Additionally, inherited traits can also play a role in the development of anxiety disorders**.**

Medical Causes

For certain individuals, anxiety might be associated with an underlying health condition. Occasionally, symptoms of anxiety serve as initial indicators of a medical illness. In such instances, a healthcare provider may recommend tests to investigate potential underlying issues. If your healthcare provider suspects that your anxiety may be medically related, they may suggest further examination.

Examples of medical issues that can be associated with anxiety include:

• Diabetes
• Thyroid problems, similar as hyperthyroidism
• Respiratory diseases, similar as habitual obstructive pulmonary complaint (COPD) and asthma
• Medicine abuse or pullout
• Elimination of alcohol, special anti-anxiety medications (benzodiazepines), or other particulars.
• Habitual pain or perverse bowel pattern
• Rare excrescences that produce certain fight- or- flight hormones.

Sometimes, anxiety can occur as a side effect of certain medications.

- You have no family history of anxiety disorders, such as a parent or sibling with the condition.
- You did not experience anxiety disorders during childhood.
- You do not avoid specific things or situations due to anxiety.
- You suddenly develop anxiety seemingly unrelated to life events, without a prior history of anxiety.

Nanosuspension

Nanosuspension refers to the colloidal dispersion of tiny drug particles in a non-aqueous medium, often water, stabilized by surfactants, polymers, or a combination of both. It is used for oral, topical, parenteral, and pulmonary administration, aiming to enhance dissolution rates and improve bioavailability due to the reduced particle size. The particle size distribution typically ranges below 1 micron, with an average size between 200-600nm. These suspensions are

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unique formulations of nanosized drug particles stabilized by suitable surfactants or polymeric stabilizers. The average particle size of pharmaceutical nanosuspensions falls within the nano range, with febuxostat nanosuspension, for instance, showing a particle size below 50 nm initially. The Ostwald-Freundlich and Noyes-Whitney equations illustrate that reducing particle size to the nanometer range can enhance drug solubility and dissolution rates [7].

In pharmaceutical discovery programs, approximately 40% of new chemical entities are poorly water-soluble or lipophilic compounds. Formulating such compounds has always posed a challenge for pharmaceutical scientists. Nano-sized particles can be employed for all drug compounds falling under biopharmaceutical classification system (BCS) classes II and IV to enhance their solubility and thereby improve gastrointestinal absorption. Micronization is commonly used for BCS class II drugs, which have good permeability but poor solubility. Various conventional methods exist to enhance the solubility of poorly soluble drugs, including micronization, solubilization using co-solvents, salt formation, surfactant dispersions, complexation, and solid dispersions. While these methods have shown some success, they are not universally applicable to all drugs, particularly those insoluble in both water and organic solvents.

Nanotechnology offers a promising approach to address the limitations of conventional methods for improving solubility and bioavailability. Nanosuspensions are preferred for compounds insoluble in water (but soluble in oil) with high log P values, high melting points, and high doses. Nanosuspension technology can also benefit drugs insoluble in both water and organic solvents. Hydrophobic drugs like Atorvastatin, Famotidine, Simvastatin, Revaprazan, and Aceclofenac are formulated as nanosuspensions. Drugs that are hydrophobic, such as aceclofenac, atorvastatin, famotidine, simvastatin, and revaprazan, are prepared as nanosuspensions. Colloidal dispersions of medication particles with a nanoscale stabilized by surfactants are called nanosuspensions[8].

Need of Nanosuspension

Furthermore, 40% of medications exhibit poor water solubility, posing challenges in formulating them into conventional tablet forms. Particularly for class II drugs with poor solubility in both aqueous and organic solvents, the issue becomes more complex. Preparation of nanosuspensions is preferred for such compounds that are insoluble in water but soluble in oil, with high log P values. While various approaches like micronization, cosolvency, solid dispersion, and cyclodextrin complexation are used to address solubility and bioavailability issues, many of these methods lack broad applicability across all drugs. In such cases, nanosuspensions are favored.

For drugs insoluble in both aqueous and organic media, nanosuspensions serve as an effective formulation approach instead of lipidic systems. They are particularly suitable for compounds with high log P values, high melting points, and high doses. Nanosuspensions can enhance the solubility of drugs poorly soluble in both aqueous and lipid media, resulting in faster attainment of maximum plasma concentration upon administration (e.g., oral or intravenous). This advantage sets nanosuspensions apart from other solubility enhancement approaches, making them valuable for molecules with poor solubility, poor permeability, or both, presenting significant challenges for formulation scientists.

• Limited bioavailability.
• Challenges in selecting the optimal formulation based on efficacy and safety.
• Variability in bioavailability due to food intake.
• Lack of proportionality between dose and response.
• Difficulty in dosing.
• Utilization of harsh excipients, such as excessive co-solvents and other additives.
• Employment of extreme pH conditions to improve solubility.

Application of Nanosuspension

- Oral Administration
- Parenteral Drug Delivery
- Ocular Drug Delivery
- Pulmonary Drug Delivery
- Drug Targeting

Passiflora incarnata

Passiflora incarnata L., also known as maypop, maracuja, or passionflower, is a plant belonging to the Passifloraceae family. It has been utilized as an anxiolytic and sedative agent since ancient times. The Passiflora genus comprises around 400 species, with some renowned for their beautiful flowers and others for their edible fruits. However, P. incarnata is the officially recognized species studied in various Pharmacopoeias. The commercial material is sourced from both wild and cultivated sources, primarily from regions such as the United States, India, and the West Indies [9].

Fig. 1. P. incarnata whole plant

Scientific Classification

- Botanical source: Passiflora incarnata L.
- Kingdom: Passifloraceae
- Subkingdom: Tracheobionta – Vascular Plants
- Superdivision: Spermatophyta-Seed plants
- Division: Magnoliophyta-Flowering plant
- Class: Magnoliopsida
- Subclass: Dilleniidae
- Order: Violales

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- Family: Passifloraceae
- Genus: Passiflora L.
- Species: Passiflora incarnata L

Morphological Characteristics

Passionflower is a herbaceous perennial vine characterized by its unique three-lobed leaves and striking flowers, which can reach lengths of 6.5 feet or more. It is commonly found along the edges of forests and fields but is becoming increasingly prevalent in many of Virginia's agricultural crops, particularly in areas where conservation tillage practices are employed.

Seedling

Cotyledons of passionflower are round, thick, and possess a pliable appearance. The initial true leaves are heart-shaped and glossy. Subsequent leaves exhibit lobes, typically three, originating from a central point (palmately lobed leaves). While seedlings may emerge, sprouts from the perennial rootstocks are more prevalent.

Roots

Initially, passionflower roots develop as a taproot, but over time, they grow into a deep perennial rootstock, from which sprouts can emerge.

Leaves

Passionflower leaves are arranged alternately along the stem, typically with minimal hairiness. Each leaf is divided into three (sometimes five) lobes that originate from a central point (palmately lobed). They are approximately as long as they are wide and are supported by petioles. At the base of the leaf blade and where the petiole meets the stem, a pair of nectar-filled glands is present.

Stems

The stems have the ability to either spread along the ground or ascend by clinging onto other plants. Typically, these stems possess a sparse covering of hair and can grow up to a maximum length of 6.5 feet.

Flowers

Individual flowers emerge from the space between the stem and leaf petioles. These flowers vary in size from 2 to 4 inches and exhibit an appealing hue of light purple to lavender.

Fig. 2. P. Incarnata flower

Medicinal Use

Insomnia

Wakefulness, or disrupted sleep, manifests as difficulty initiating or sustaining sleep, resulting in a fragmented or superficial rest. This symptom leads to issues with both the duration and depth of sleep, and chronic wakefulness is linked to conditions such as headaches, depression, anxiety disorders, and other health issues. Historically, Passiflora incarnata L has been utilized as a remedy for addressing wakefulness**.**

Sedation

In ultramodern societies, there's a higher prevalence of mental exertion compared to physical labor, leading to a significant rise in internal stress. Over time, chronic stress can lead to various internal conditions such as anxiety, restlessness, and unease, as well as physical ailments. Passiflora incarnata L. has a longstanding history of use as a remedy for anxiety, with people worldwide utilizing it for this purpose. Research on its sedative effects has been conducted using mice. In one study, mice were divided into groups of ten, with four groups receiving pentylenetetrazol (PTZ; 90 mg/kg i.p.) 30 minutes prior to administration of Passiflora incarnata L. at doses of 0.05, 0.2, and 0.4 mg/kg i.p., prepared from leaves, flowers, and fruits.

Menopause Symptoms

As women age, the function of the ovaries weakens due to a decline in estrogen production, leading to the onset of menopausal symptoms. Menopause refers to the period following the last menstrual cycle. While the timing of these changes varies from person to person, they typically begin gradually in the middle to late stages of life. Postmenopausal women may experience heightened levels of depression, anxiety, and stress, which can be influenced by their overall health status related to reproductive functions. Physical symptoms of menopause include back pain, night sweats, itchy skin, osteoporosis, hot flashes, and internal feelings of anxiety. More than 50% of menopausal women experience one or more of these symptoms. A clinical study compared the effects of Hypericum perforatum and Passiflora incarnata L. on menopausal symptoms.

Anti-inflammatory and Antioxidant

Herbal remedies are known for their diverse therapeutic properties, including anti-allergic, antioxidant, anti-inflammatory, antiviral, anti-proliferative, and anti-carcinogenic effects. Passiflora foetida, a type of passionflower, was investigated for its analgesic and anti-inflammatory properties using an ethanol extract in writhing and hot plate tests. Furthermore, the anti-inflammatory effects of carrageenan were evaluated for their impact on histamine-induced rat paw edema. The results showed that a dose of 200 mg/kg of Passiflora foetida extract significantly reduced pain sensation (13.50 ± 0.43 seconds) within 20 minutes using the hot plate test in mice. A dose of 100 mg/kg of the ethanol extract demonstrated significant anti-inflammatory effects in rats (1.302 ± 0.079). Passiflora foetida exhibits notable analgesic and anti-inflammatory properties.

Anti-tissue activity

In one study, mice given sulfur dioxide to elicit a cough. The methanolic extract of Passiflora incarnata (100 and 200 mg/kg by oral method) shown strong antitussive action, similar to that of codeine phosphate (10 and 20 mg/kg by oral route, respectively).

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II. MATERIALS AND METHODOLOGY

Materials

Passiflora Incarnata

- The crude herbal drug leaf of Passiflora incarnata was gathered from the Botanical Garden PDEA'S SGRS College of Pharmacy, Saswad, Pune.
- The extraction method employed was Soxhlet Extraction.
- The resulting extract utilized for further study was the methanolic extract of Passiflora incarnata.

Experimental Animal:

Male Swiss Albino Mice weighing 20-25g were procured from the animal house of PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad. These mice were housed in the same animal facility at the college. The mice were between 4-5 weeks old. The experimental protocol with the number SGRS/IAEC/04/2021-22 was approved by the Institutional Animal Ethics Committee (IAEC) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Methodology

Preparation of Passiflora incarnata leaves extract

The leaves of P. incarnata were gathered from the Botanical Garden of PDEA Seth Govind Raghunath Sable College of Pharmacy, Saswad, Purandar, Pune. These collected leaves underwent washing with double-distilled water, followed by drying under shade and grinding into a fine powder. The plant powder was then defatted using n-hexane in a Soxhlet extractor for a duration of 6-8 hours. Subsequently, for the extraction of polyphenolic and flavonoid contents, the defatted plant powder was subjected to extraction with ethanol (95%, HPLC grade) using a Soxhlet extractor. The resulting extract was concentrated using a rotary evaporator and stored in a refrigerator for future use.

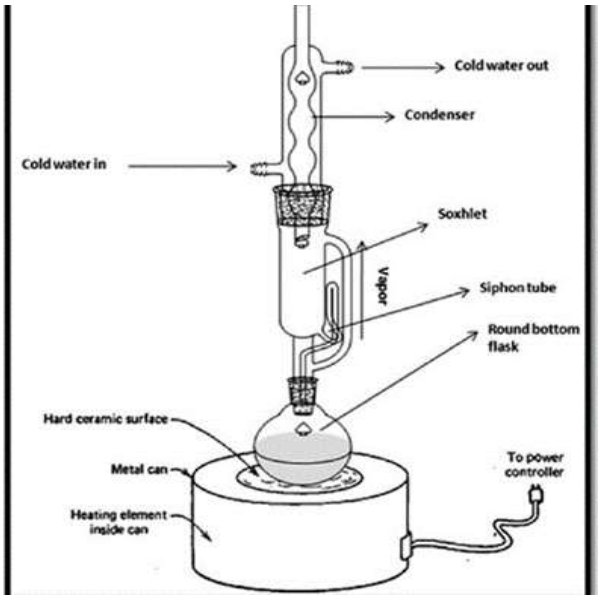

Fig. 3. Soxhlet Apparatus

1. Place the sample material containing the desired compound into the thimble.
2. Insert the thimble loaded with the sample into the main chamber of the Soxhlet extractor.
3. Add the chosen solvent to the round bottom flask and position it on a heating mantle.
4. Connect the Soxhlet extractor above the round bottom flask.
5. Attach a reflux condenser above the extractor, ensuring that cold water enters at the bottom and exits above.
6. Once the apparatus is ready, bring the solvent's temperature to reflux and let it extract for the required quantity of time.

Fig. 4. Plant Extract

Formulation of Nano suspension

The nano-precipitation approach was employed for the formulation of the nanosuspension medication. Briefly, the plant extract (1 g) was completely dissolved in ethanol (10 mL) as the organic phase and filtered. The prepared organic phase was then gradually added (1 mL/min) into a 100 mL anhydrous solution of stabilizer (polysorbate-80, 2% v/v) with constant mechanical stirring (using a Lab Mechanical Stirrer JJ-1, China) at 6000 rpm for approximately 6 hours at room temperature. For comparative analysis, a coarse suspension of P. incarnata was prepared by dissolving the plant extract (1 g) in distilled water (100 mL) at room temperature.

Fig. 5. Organic Solvent

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Fig. 6. Mechanical Stirrer

Characterization

UV- Visible Spectra

1. UV-Vis spectra were obtained using a JASCO V-630 spectrophotometer.
2. A quantity of 10 mg of the drug was dissolved in 10 mL of distilled water.
3. Distilled water was utilized as a blank, and the wavelength was verified within the range of 400-600 nm.

UV data

UV data was conducted by using JASCO V-630. The dilution for drug was prepared 1mg/ml by using distilled water. The wavelength observed for the drug was 410nm.

Fig. 7. Nanosuspension

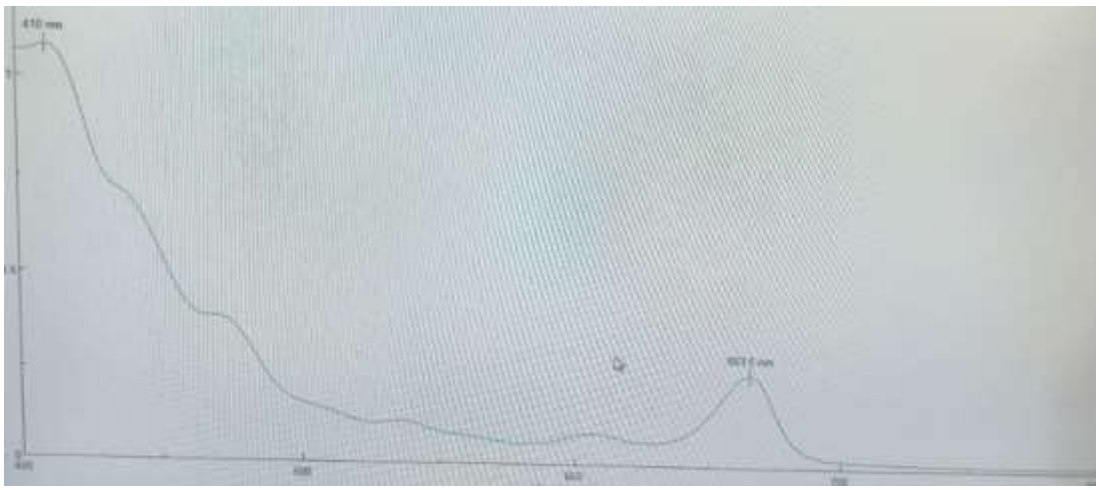

Fig. 8. Absorption Spectra of UV

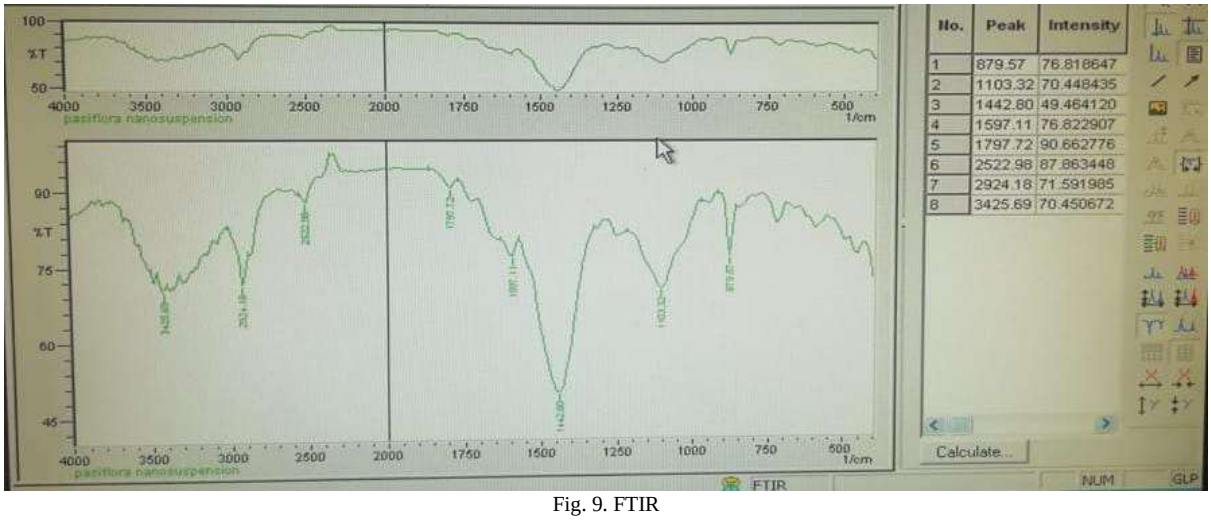

Fig. 9. FTIR

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FT-IR (Fourier Transformed Infrared Spectroscopy) Spectra:

1. FTIR data was acquired using a SHIMADZU FTIR 8400S spectrophotometer.
2. The sample was triturated with potassium bromide at a ratio of 1:100.
3. Potassium bromide was employed as the blank/reference.
4. FTIR spectra of the provided herbal drug were then recorded within the range of 400-4000 cm^-1.

IR data

IR data was conducted by using SHIMADZU FTIR-8400S.The drug was triturated with potassium bromide [reference standard] and IR was observed.

Functional Group	Reference Value	Observed Value
OH	3700-3584	3425.69
C=O	1800-1600	1597.11
O-C=O	1750-1735	1797.72
C-H	3100-3000	2924.18
C=C	1650-1566	1442.80

Animal Modelling

The use of animals for scientific purposes has been a longstanding practice in both natural exploration and drug development, yet it remains a topic of frequent debate in our societies. The striking anatomical and physiological similarities between humans and other creatures, especially mammals, have led researchers to investigate a wide range of mechanisms and test new treatments in animal models before applying their findings to humans. However, it's important to note that not all results obtained in animals can be directly extrapolated to humans, a point emphasized by those who question the value of animal research.

The ethical implications of using animals for human purposes are frequently debated, particularly concerning the rights of animals and the potential for harm. These issues are often intertwined in complex arguments, making it challenging for the general public and policymakers to form clear perspectives on the matter.

Humans and other mammals are highly complex organisms with organs that perform diverse physiological functions in a coordinated manner. Biological interactions involve a complex network of hormones, circulating factors, cells, and cross-talk between cells in various tissues [9].

Experimental Analysis (In vivo Model)

A. Elevated plus Maze study in Mice:

The elevated plus maze test is a valuable method for studying anxiety-related behavior in rodents. This apparatus consists of two open arms and two closed arms arranged in a plus-shaped maze, elevated 50 cm above the floor. The open arms and closed arms have dimensions of 50×10×40 cm and feature an open roof arrangement.

Swiss albino mice are among the most commonly used outbred mice strains. However, Swiss mice sourced from different origins may exhibit significant variations due to long-term separation from the original stock. This is also true for Swiss albino mice bred at the Institute Torlak for over 80 generations. These mice have been extensively utilized in toxicology studies involving vaccine production and as general-purpose mice in various fields of research. The biochemical, physiological, genetic, and immunological characteristics of these mice have been thoroughly investigated over time, providing a solid foundation for the data presented in this paper.

The natural characteristics described in this paper offer valuable insights into the potential use of this mouse strain in various studies conducted at the Torlak Institute and other institutions focused on investigating different biological and biomedical phenomena.

Animal models have played a crucial role in addressing a wide range of scientific questions, from basic understanding to the development and evaluation of new vaccines or treatments. The use of animals is not only based on the extensive biological similarities among most mammals but also on the fact that many human conditions also affect other animal species. This is particularly true for infectious diseases and common conditions such as Type I diabetes, hypertension, allergies, cancer, epilepsy, and myopathies. Furthermore, the mechanisms underlying these conditions are often so similar that approximately 90% of veterinary medicines used to treat animals are identical or very similar to those used in humans [10].

1. *Procedure*

- The mice were placed in the testing room one hour prior to testing to minimize the effects of stress on their behavior during the test.
- Test drugs were administered orally one hour before testing.
- The behavior of the animals was recorded using a maze controller and a camera positioned over the elevated plus maze.
- Each mouse was placed at the center square area of the maze facing towards one of the open arms.
- The mice were allowed to move freely between the open and closed arms for the next 5 minutes.
- After 5 minutes, the animals were removed from the maze and returned to their original cages.

Fig. 10. Animal Dosing

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Fig. 11. Process for Elevated Plus Maze

2. Computerized analysis

The elevated plus maze (EPM) was recorded using a high-resolution video camera, WVCP244, manufactured by Panasonic in Secaucus, USA. Video analysis was conducted using Top Scan, a software program for animal behavior analysis, specifically Top View Animal Behaviour Analyzing System (version 1.00), developed by Clever Sys Inc. in Preston, USA. The analysis was performed by an impartial and treatment-blinded individual.

3. Animals

Swiss Albino Mice aged between 6 and 12 weeks and weighing 20–25 g were obtained from PDEA Seth Govind Raghunath Sable College of Pharmacy in Saswad, Pune, Maharashtra. The mice were housed in cages of 5 each at a temperature of 20 ± 1°C in a 12-hour light/dark cycle. They had access to tap water and food pellets ad libitum. Groups of 10 mice were randomly allocated to different treatment groups and tested in varying orders. The animals underwent repeated testing under consistent experimental conditions. All experiments were conducted in a quiet room with controlled lighting conditions between 8:00 a.m. and 1:00 p.m.

All animal housing and experimental procedures were conducted in accordance with the policies and guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Florida, Gainesville, USA.

4. Results

• The Elevated Plus Maze Model revealed that the mice exhibited a significant preference for the open arms, indicating reduced anxiety-like behaviour.

• Administration of an anxiolytic agent (Passiflora incarnata Nano suspension) resulted in an increase in the frequency of entries into the open arms and prolonged the time spent in the open arms of the Elevated Plus Maze, further indicating its anxiolytic effects.

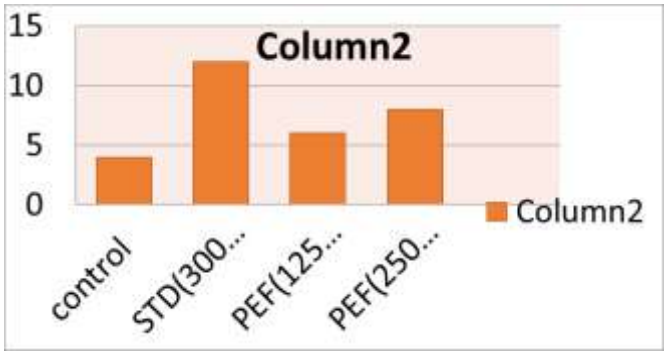

Fig. 12. Comparison of Standard and Control Formulation of Elevated Plus Maze Study

B. Marble Bury Test study in Mice:

The Marble Burying test serves as an important model for assessing anxiety, neophobia, and obsessive-compulsive behaviour in rodents. This test is increasingly utilized in screening potential treatments such as antidepressants, antipsychotics, anxiolytics, and antianxiety drugs.

Neophobia refers to a fear of unfamiliar or novel objects, which is observed in rodents when they encounter any new or strange object. Novel marbles, acting as unfamiliar objects, can induce feelings of unease or fear in rodents, prompting them to engage in digging and burying activities. This burying behaviour is stimulated by the presence of noxious objects.

Antianxiety drugs are expected to reduce fear or anxiety towards these marbles, resulting in a decrease in the number of marbles buried by the rodents.

Swiss albino mice are commonly used in such experiments. However, Swiss mice from different sources may exhibit considerable variations due to long-term separation from the original stock. This is also true for Swiss albino mice bred at the Institute Torlak for over 80 generations. They have been extensively used in toxicology studies related to vaccine production and as general-purpose mice in various fields of research.

The biochemical, physiological, genetic, and immunological characteristics of these mice have been thoroughly investigated over time, providing a solid foundation for the data collected and presented in research papers. This understanding of their natural traits offers valuable insights into the potential use of this mouse strain in studies conducted at the Torlak Institute and other institutions focused on exploring various natural and biomedical phenomena.

1. Procedure

• Standard cages were filled to a depth of approximately 5cm with sawdust bedding, which was lightly tamped down to create an even and flat surface.

• Ten glass marbles were evenly spaced and arranged in a regular pattern on the bedding surface.

• Mice were administered the drug orally and evaluated after 1 hour of oral treatment.

• Each cage contained one mouse, which was allowed to freely explore the cage for 30 minutes.

• The experiment was conducted in a quiet environment to minimize disturbances.

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• After 30 minutes, the mice were carefully removed from the cages and returned to their original housing.
• The number of marbles buried in each cage was recorded as an indicator of anxiety-like behavior.

Fig. 13. Animal Dosing

Fig. 14. Marble Burry

2. Computerized analysis

The Elevated Plus Maze (EPM) was recorded using a high-resolution video camera model WVCP244 manufactured by Panasonic in Secaucus, USA. Subsequently, the analysis of the recorded videos was carried out using Top Scan, which is part of the Top View Animal Behaviour Analysing System (version 1.00) developed by Clever Sys Inc. in Preston, USA. The analysis was conducted by an impartial individual who was blinded to the treatment conditions, ensuring unbiased results.

3. Animals

Swiss Albino Mice aged between 6 and 12 weeks and weighing 20–25 g were housed at PDEA Seth Govind Raghunath Sable College of Pharmacy in Saswad, Pune, Maharashtra. They were accommodated in cages of 5 mice each at a temperature of 20 ± 1°C with a 12-hour light/dark cycle. These mice had ad libitum access to tap water and food pellets.

For experimental purposes, groups of 10 mice were randomly assigned to different treatment groups and tested in varying sequences. The animals underwent repeated testing under consistent experimental conditions.

All experiments were conducted in a quiet environment under controlled lighting conditions between 8:00 a.m. and 1:00 p.m.

All animal housing and experimental procedures adhered to the policies and guidelines set forth by the Institutional Animal Care and Use Committee (IACUC) of the University of Florida, Gainesville, USA**.**

4. Results

The Marble Bury Test results indicate that prior to administration of Passiflora incarnata nanosuspension, mice buried 8 out of 10 marbles, indicative of anxiety-related behavior. However, following the administration of Passiflora incarnata nanosuspension, the frequency of buried marbles decreased to 6 (at 125 mg/kg dose) and 5 (at 250 mg/kg dose). This decrease in the number of buried marbles suggests a reduction in anxiety-related behavior, indicating the potential antianxiety activity of Passiflora incarnata.

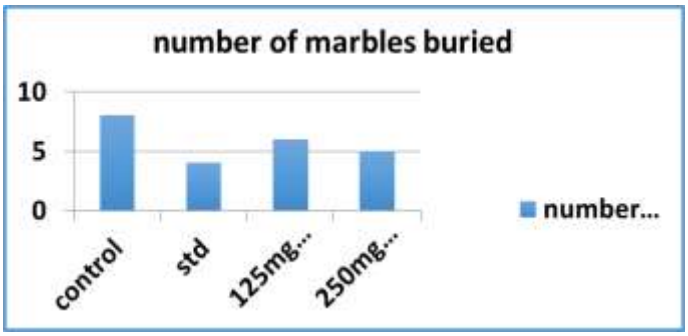

Fig. 15. Comparison of Standard and Control Formulation of Marble Burr

III. CONCLUSION

The Passiflora Incarnata Nanosuspension demonstrated a pronounced anti-anxiety effect in both the Elevated Plus Maze (EPM) and Marble Bury Test (MBT). In the EPM, it significantly increased the number of entries into the open arm and the percentage of time spent in the open arm, indicating reduced anxiety-like behavior. Similarly, in the MBT, the nanosuspension reduced anxiety or fear by decreasing the number of marbles buried by the mice. Among the treatment groups, Group 2 receiving a dose of 250 mg/kg exhibited the

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most notable anti-anxiety effect in both models. This suggests that the 250 mg/kg dose of Passiflora Incarnata Nanosuspension is particularly effective in alleviating anxiety-related behaviours in mice.

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