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PROMOTING SLEEP HEALTH WITH HERBAL TEA: DEVELOPMENT, EVALUATION, AND ANXIOLYTIC EFFECTS

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Grant Support Declaration
I, Mehwish Khan, hereby declare that the research study titled ""Promoting Sleep Health with Herbal Tea: Development, Evaluation, and Anxiolytic Effects" has been conducted with the financial support provided by Jinnah University for Women, Karachi, Pakistan.
This research project was carried out as part of my affiliation with Faculty of Pharmacy, Department of Pharmacognosy at Jinnah University for Women. The funding received from the university played a vital role in facilitating the following aspects of the research:

1. **Research Design and Data Collection:** The support from Jinnah University for Women enabled the development of the research design, data collection, and data analysis phases of this study.
2. **Laboratory Facilities:** The university provided access to state-of-the-art laboratory facilities, equipment, and resources necessary for conducting experiments and analyses related to this research.

I wish to express my sincere gratitude to Jinnah University for Women for its generous support, which has made this research endeavor possible. The funding has significantly contributed to the advancement of knowledge in the field of Pharmacognosy, and I am appreciative of the university's commitment to promoting research and academic excellence.
Furthermore, I would like to acknowledge that all aspects of this research were conducted in accordance with ethical guidelines and research protocols, and any potential conflicts of interest were appropriately disclosed.

Should you require any additional information regarding the grant support provided by Jinnah University for Women for this publication, please do not hesitate to contact me at mehwish.rph@gmail.com
Sincerely,

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ABSTRACT:
Insomnia, a widespread issue in modern life characterized by sleep difficulties, has prompted interest in herbal remedies known for their calming effects. This study explores the potential of a herbal tea formulation containing Passiflora incarnata L. (PI), Valeriana officinalis (valerian), and Chamomilla matricaria extracts as a solution for insomnia. Microscopic examination validated the authenticity of the herbal components, while extracts were prepared through meticulous maceration and evaporation. Safety analysis, assessing lethal dose (LD50), confirmed the tea's safety with an LD50 of 2400 mg/kg. In an animal study involving thiopental sodium-induced sleep in mice, significant findings emerged. The test group receiving the herbal tea at 300 mg/kg and 500 mg/kg exhibited notably shorter sleep onset times ($p < 0.05$) compared to the control group. Additionally, their sleep duration at these doses significantly exceeded that of the control group ($p < 0.01$). These results collectively suggest that the herbal tea, especially at doses of 300 mg/kg and 500 mg/kg, exhibited an anxiolytic effect, as evidenced by increased open-arm exploration and reduced anxiety-like behavior in the Elevated Plus Maze (EPM) test. These findings highlight the herbal tea's potential as a safe and effective sleep aid, offering promise in addressing insomnia. However, further research is necessary to explore its clinical applications in humans, advancing our understanding of its broader therapeutic potential for sleep-related issues.

Keywords: Sleep, Anxiety, Toxicity, Microscopic evaluation, Herbal Tea.

1. INTRODUCTION:

A sleep problem called insomnia, commonly referred to as sleeplessness, is characterised by trouble falling asleep or remaining asleep for the appropriate amount of time, which has a detrimental effect on quality of life[(1)]. Insomnia can be caused by a number of things, such as stress, illness, and noise[(2)].Chronic insomnia can cause immune system alterations, memory issues, slower reaction times, emotional difficulties, and slower reaction times[(3)].Numerous medications, including benzodiazepines, non-benzodiazepines, antidepressants, and antihistamines, are frequently used to treat insomnia. Widely used as hypnotics, benzodiazepines and benzodiazepine-related substances can have unfavourable side effects include drug dependence, tolerance, rebound insomnia, and amnesia [(4)]. Higher doses could be needed to maintain efficacy because of the onset of tolerance [(5)]. As a result, research is ongoing to find novel hypnotic substances that are more effective and have fewer negative effects[(6)].In order to create new medicines to treat a variety of disorders, herbal compounds have long been investigated[(6, 7, 8)].Traditional uses for the herb Valeriana officinalis, or valerian, include sedation and assistance in falling asleep. It is thought to soothe the nervous system and encourage relaxation. Although valerian is frequently used as a natural sleep aid, there is conflicting scientific data to support its efficacy. The effects of valerian on sleep and insomnia have been the subject of numerous investigations. In 2023, the effects of valerian intervention on subjective and objective sleep indices were evaluated in people with insomnia, according to a comprehensive review and meta-analysis published in the journal Current sleep medicine. 1433 participants from 21 randomised controlled trials were included in the study. In summary, valerian therapy for insomnia may result in improvements in the quantity, quality, and duration of the subjective sleep as well as in some of the objective sleep indices, particularly the length of the NREM stage(9). The effects and

mechanisms of valerian volatile oil in treating insomnia were examined in a 2022 study that was published in the journal Frontiers in Nutrition. The researchers discovered that valerian oil, specifically its active ingredient caryophyllene, improved serotonin activity by upregulating the 5-HT1AR receptor and enhancing serotonin release. They did this using transcriptome sequencing and network pharmacology. G protein-coupled receptors and the transformation of ATP into cAMP were implicated in this interaction. Additionally, serotonin and GABA expression were elevated as a result of the protein kinase PKA activating the serotonergic synaptic signal pathway, lengthening sleep duration and lowering tension and anxiety brought on by insomnia. These results shed important light on the possible therapeutic uses of valerian volatile oil for treating insomnia[10].German chamomile, also known as chamomile matricaria, is well known for its sedative and relaxing properties. There are many research-based arguments in favour of chamomile flowers' clinical efficacy in treating insomnia. In a 2017 study that was published in Complementary Therapies in Medicine, the effects of chamomile extract on older people's sleep quality were examined in a single-blind, randomised controlled trial. Sixty elderly residents of an Iranian day care nursing home who were at least sixty years old were split into a treatment group and a control group at random. The control group received wheat flour capsules in the same manner as the treatment group, which received chamomile extract capsules twice daily for a total of 28 days. Before, during, and after the intervention, sleep quality was evaluated using the Pittsburgh Sleep Quality Index. The outcomes revealed that the treatment group's sleep quality greatly improved as compared to the control group. Therefore, the study came to the conclusion that chamomile extract can be a secure and useful choice for improving elderly people's sleep[11]. A plant from the Passifloraceae family called Passiflora incarnata L. (PI), which is widely used in South America, has demonstrated benefit for anxiety and sleep. The effectiveness of it as a prescribed sleep aid, however, has received little testing. This study used rat glioma cells and experimental animals to examine an extract made from PI. After being given to the animals orally, the PI extract dramatically enhanced immobility time and palpebral closure time in the cells and decreased GABA receptors in the cells. Melatonin levels in the blood rose as well. These results imply that PI extract has sleep-inducing properties and may be used to treat human insomnia[12]. Due to their natural components' calming, muscle-relaxing, and sleep-regulating qualities, herbal teas are advantageous and useful for sleep and numerous cures. Herbs including chamomile, valerian root, and passionflower ease tension in the body and mind and promote relaxation and better sleep. Herbal teas are a safe and sustainable choice for managing sleep concerns, increasing general wellbeing, and assisting digestion in addition to their non-habit-forming nature and mild effects. Although individual results may vary, integrating premium herbal teas into a nighttime regimen might help you get a better night's sleep that will leave you feeling refreshed the next day. Because of these mentioned herbs having evidence on sleep promoting effects we have designed a novel herbal tea to evaluate the anti-anxiety and sleep-prolonging effect as well as its microscopical characters and their toxicological studies. The objective of this study is to determine whether a herbal tea formulation including extracts from Passiflora incarnata L. (PI), Valeriana officinalis (valerian), and Chamomilla matricaria can treat insomnia. The goal is to use an animal model to evaluate the efficacy and safety of this herbal tea in enhancing sleep characteristics, such as sleep onset time and sleep length. Through behavioural tests, notably the Elevated Plus Maze (EPM) test, the study also attempts to analyse the anxiolytic effects of the herbal tea. With the potential for future therapeutic uses in people to treat insomnia and related sleep difficulties, the ultimate goal is to present scientific evidence demonstrating the safety and efficacy of herbal tea as a sleep aid. We have used IBM SPSS Version 20 for these research study. For post hoc comparisons; we used the one-way analysis of variance (ANOVA) in conjunction with the Bonferroni test. When conducting several pairwise comparisons across different treatment groups in our experimental mouse study, the Bonferroni test is a useful statistical tool for reducing the chance of Type I errors. It allows us to detect statistically significant differences between the groups under inquiry while efficiently correcting for the inflation of the overall Type I error rate. Using the Bonferroni test, we were able to identify the individual treatment groups that had true and statistically significant differences in the assessed outcomes. This rigorous statistical approach ensures the

reliability and validity of our findings and helps us to draw meaningful inferences from our research data.

2. METHODS:

2.1. Reagents/Drugs:

Distilled water, chloral hydrate (10%), glycerin (50%) and iodine (5%) Thiopental sodium injection Inj 500mg (Abbot laboratories Pvt Ltd), Trazodone Hcl 50 mg from Adamjee Pharmaceutical Pvt Ltd Karachi.

2.2. Collection of plant materials and extraction:

Roots of Valerian officinalis, Flowers of Chamomile matricaria and leaves of Passiflora incarnata were procured from botanical shops and nurseries near Karachi (24.8607°N 67.0011°E). The identity of these herbs was certified and confirmed by Dr. Beena Naqvi, Principal Scientific Officer of the Pakistan Council of Scientific and Industrial Research (PCSIR). Chamomile, Passiflora incarnata, and Valerian officinalis have herbarium voucher numbers of FMRC/Herb./0061/22, FMRC/Herb./0050/22, and FMRC/Herb./0060/22, respectively, and were placed in the PCSIR Herbarium in Karachi. The maceration method was used in the extraction process, with 50 g of each herb treated to two extractions with 500 ml of ethanol over a week, with periodic agitation in order to ensure full extraction of soluble components. The solvent of choice was ethanol. The herbs were once more macerated with ethanol for a further 3 days after the extract had been filtered with whatman filter paper. The extracts were once again filtered, combined with the prior extracts, and then evaporated in a rotary evaporator. Extracts were evaporated, and leftovers were then placed in a fume hood to continue drying. After that, all of the dried extracts were put into glass jars and labelled with the names VAL (Valerian officinalis), CHM (Chamomile), and PSF (Passiflora incarnata).

2.3. Microscopic evaluation:

With the use of chemical reagents including chloral hydrate (10%), glycerin (50%) and iodine (5%), the powders of Valerian officinalis, Chamomile Matricaria and Pasiflora incarnata were examined microscopically using an Olympus electronic microscope at a magnification of (10X). Powder microscopy images were captured using a digital camera with the model number M863 (B).

2.4. Experimental Animal:

Male and female Swiss albino mice were used in the study. These animals (25g each) were grouped separately in plastic boxes in the animal house facility of faculty of pharmacy, Jinnah University for women, Karachi at temperature of 20 ± 2°Cand recurring cycle of 12 hrs of light and dark periods.

2.5. Ethical review board approval:

The Jinnah University for Women Institutional Ethical Review Board examined and authorised all experimental methods and letter JUW/IERB/PHARM-ARA-018/2022 was issued.

2.6. Experimental design:

2.6.1. For Lethal dose determination:

Nine groups with two mice each were created for the purpose of determining the lethal dose (LD50) of herbal tea. Interperitoneally, the herbal tea extract was given to Groups 1–8 at doses of 25, 50, 100, 200, 400, 800, 1,600, and 3,200 mg/kg, while Group 9 received normal saline as a carrier. For a period of 24 hours, the death or mortality rate was observed and reported. Both the lowest dose, which killed one animal, and the highest dose, which did not kill any mice, were noted. The median fatal dose was determined to be the mean of these two doses(13, 14, 15, 16) .

2.6.2. Thiopental sodium induced sleep test:

To assess the sedative property of herbal tea a previously disclosed approach was slightly modified on mice's thiopental sodium-induced sleep duration. Swiss albino mice were divided into four groups,

each with four mice. The standard group received trazodone HCL (at a dose of 0.36 mg Kg-1oral) while the test group 1 received herbal tea (at a dose of 300 mg/kg body weight, orally). The test group 2 received herbal tea (at a dose of 500 mg/kg body weight, orally). Then, 40 mg/kg of intraperitoneal thiopental sodium was administered to each animal 20 minutes later. Then, 40 mg/kg of intraperitoneal thiopental sodium was administered to each animal 20 minutes later. Following that, the animals were monitored for the latent period (the interval between receiving thiopental and losing the righting reflex) and sleep length (the interval between losing and regaining the righting reflex)(17, 18).

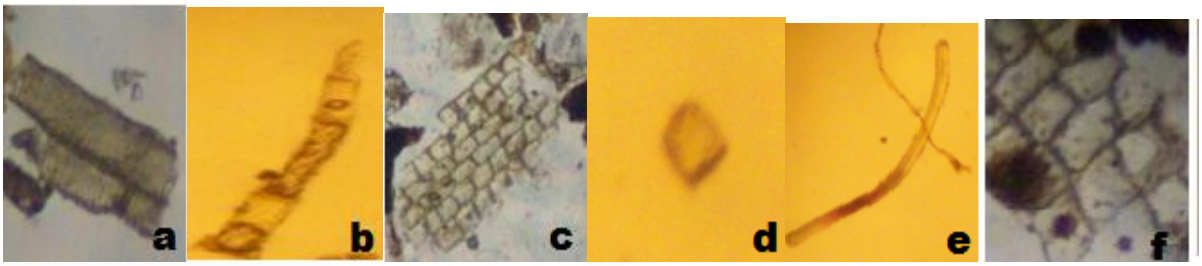
2.6.3. Elevated plus maze test:

The elevated plus maze test, which is frequently used to measure anxiety-based behaviour in rodents, involves placing the animals on an apparatus made up of two open and two closed extensions connected by a central path, all of which are elevated above the ground, typically at a height of around one metre. To conduct the test, 4 groups of mice each group containing 4 swiss albino mice.Control group treated with Distilled water,Standard group with Trazodone Hcl with dose of 0.36 mg/Kg ,One group of mice received herbal tea of dose 300 mg/kg and the other group treated with 500 mg /Kg.One mice from each group are placed in the centre of the device and given 5 minutes to roam around freely after a particular amount of time has passed after the administration of a standard or test drug. The number of admissions into the open and closed arms, as well as the amount of time spent in each arm, are manually counted during this period. These parameters assist assess the impact of the drugs being tested and shed light on the animal's anxiety-related behaviour[19].

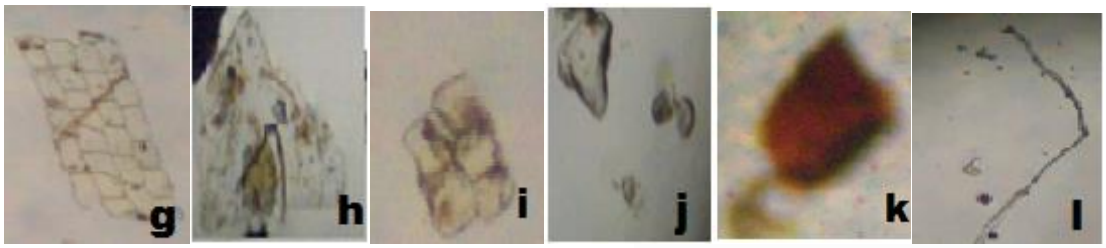
3. RESULT:

3.1. Microscopical evaluation:

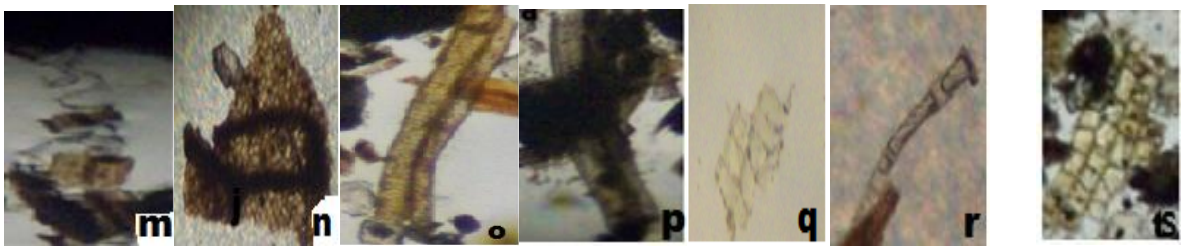
Different diagnostic features were observed after the powdered microscopic studies of root of valerian officinalis(blackish brown colour),Leaves of Passiflora incarnate(greenish brown color) and Floral parts of Chamomile matricaria(brown colored powder) see fig (1,2,3).



a)Reticulatexylem vessels b)unicellular trichome c)cork cells d)prismatic crystal of calcium oxalate e)Phloem fibers f)Parenchymatous cells filled with starch grains

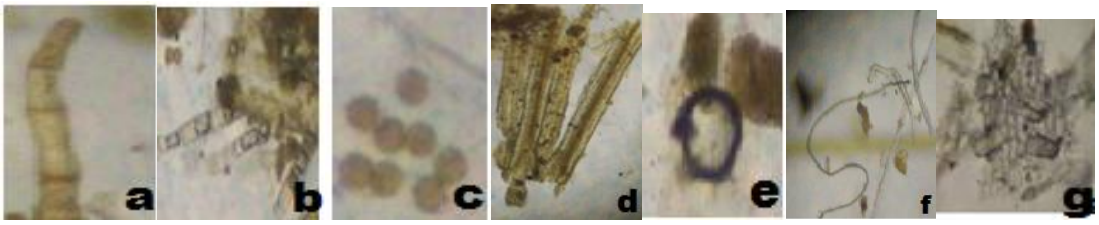


g)Laticiferous vessels attached with epidermal layer h)polygonal cellulosic oil globules and aleurone grains i)Rosette crystal of calcium oxalate j)Calcium oxalate crystals k)Tannin content l) Epidermal fiber

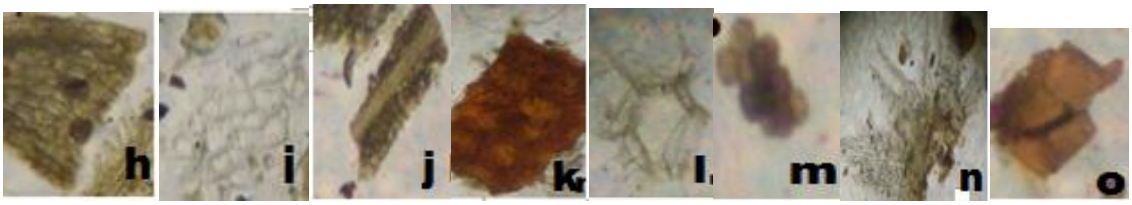


m) conducting tissues n)lignified tissues o) meta-xylem vessels p) lignified vessels q)Spongy cells r)Multicellular sharp hair s) epidermal cells filled with starch grains

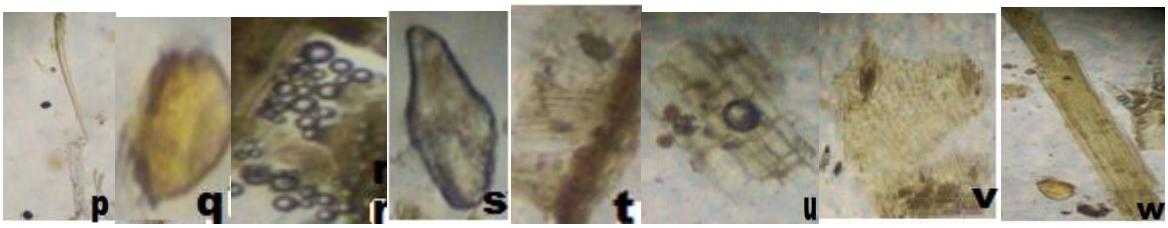
Figure 1 : Microscopic evaluation of *Valerian Officinalis*



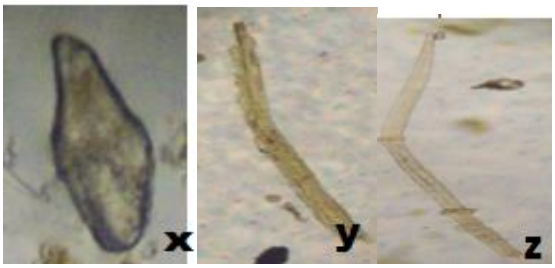
a)Multicellular trichome b)Covering trichome c)Scattered pollen grains d)Oil cell g) Forming trichomes on epidermal layer



h)schizogenous oil gland i)parenchyma j)Vascular tissues k)Lignified cork cells l)Cortical cell m)Group of pollen grains n)funiculus trichome o)Calcium oxalate crystal

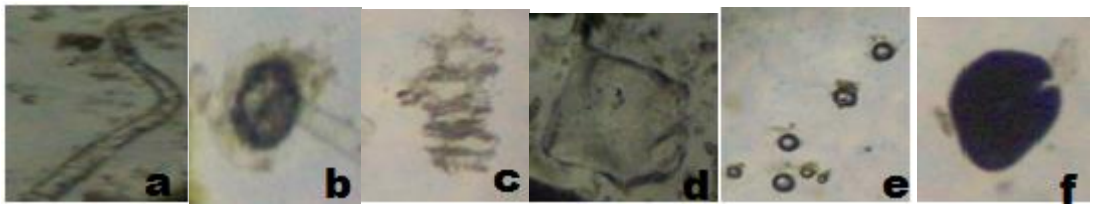


p)Unicellular trichome q)oil cell r)Group of oil cells s)sclereids t) Annular vessel element u)parenchyma tissue v)parenchyma tissue with oil gland w)Columnar sclereid

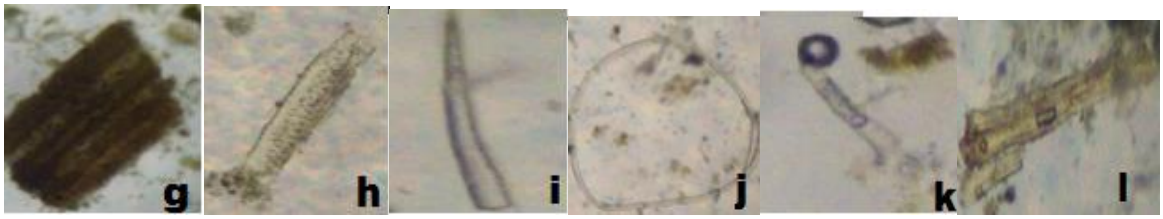


x) Cylindrical sclereid y)Septal fiber z)Glandular trichome

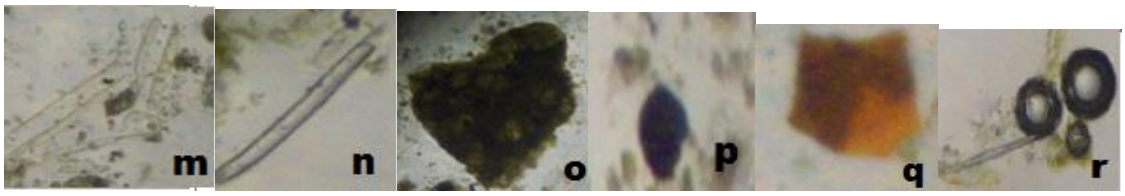
Figure 2 : Microscopic evaluation of *Chamomile matricaria*



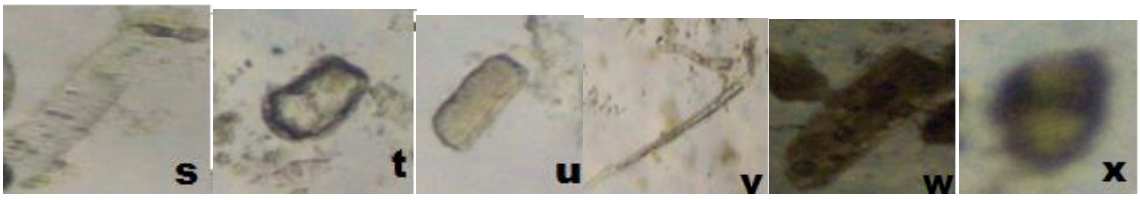
a)Trichome b)Stomata c)Parenchymatous cells d)square crystal of calcium oxalate e)Pollen grains f)Part of ovary



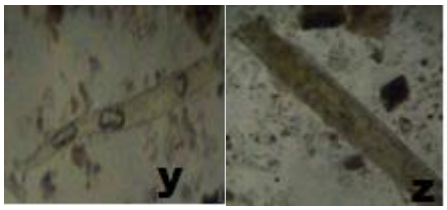
g)Xylem vessels with spiral thickening h)Xylem vessels with reticulate thickening i)unicellular trichome j)lignified fiber k)Capitate trichome l)Thick walled tracheids



m)Group of fibers n) Unicellular trichome o)epidermal cells p)starch grain q)Prismatic crystal of calcium oxalate r) Group of Stomata



s)Xylem vessels with spiral thickening t)Glangular cell u)rectangular crystal of calcium oxalate v)uniseriate trichome w)epidermal cells x)fragment of epidermal cell



y)Long trichome z)vessel

Figure 3 : Microscopical Evaluation of *Passiflora incarnata* leaves

1.1. Lethal dose (LD50) or toxicity assesments:

The lowest dose, which resulted in the death of one mouse, was 3200 mg/kg, whereas the maximum dose, 1600 mg/kg, had no effect on the mortality of any animals. The LD50 was calculated as the mean of these two dosages (2400 mg/kg).

1.2. Thiopental sodium induced sleeping time test:

After 20 mins of administration of test samples each animal was injected intraperitoneally, thiopental sodium (40mg/kg) and the sleeping time was noted by recording the interval between the loss and regaining of righting reflex. We have observed that the time of onset of sleep and duration of sleep of all groups.The results showed that the time of onset of sleep of Test group (300mg/kg) and (500

mg/kg) group are significant as compared to control while Trazodone Hcl (0.36mg/kg) showed moderate results with respect to onset of sleep as well as duration of sleep (P≤0.05 by SPSS One way Anova Bonferoni test).The duration of sleep is more significant in Test group 500mg/kg and 300 mg/kg(P≤0.05 by SPSS One way Anova Bonferoni test).

Table 1: Results of sleep onset and duration in Thiopental sodium induced mice in test and standard group w.r.t control group

Treatment	Dose mg/kg orally	Responses	
		Onset of sleeping (mins)	Duration of sleeping(mins)
C	0.5ml(d.w)	10.750±.47871	79.2500±4.11045
Test Group1	300mg/kg	4.500±.28868*	145.0000±5.40062**
Test Group 2	500mg/kg	3.000±.40825**	171.0000±2.48328**
TZD	0.36mg/kg	5.125±.42696*	114.000±4.60072*

Values are presented as Mean+ S.E.M; (n = 4); P≤0.05* Significant with respect to control (*= Significant results; **= Highly Significant results) One way Anova followed by Bonferroni's test.

1.3. Elevated plus maze apparatus:

In the Elevated Plus Maze (EPM) test, the control group showed moderate anxiety-like behavior, with balanced entries into open and closed arms. Trazodone HCl reduced anxiety, leading to more open arm exploration. Herbal tea at 300 mg/kg and 500 mg/kg significantly reduced anxiety, indicated by increased open arm entries and longer time spent in the open arm, suggesting strong anxiolytic effects(P≤0.05 by SPSS One way Anova Bonferoni test).

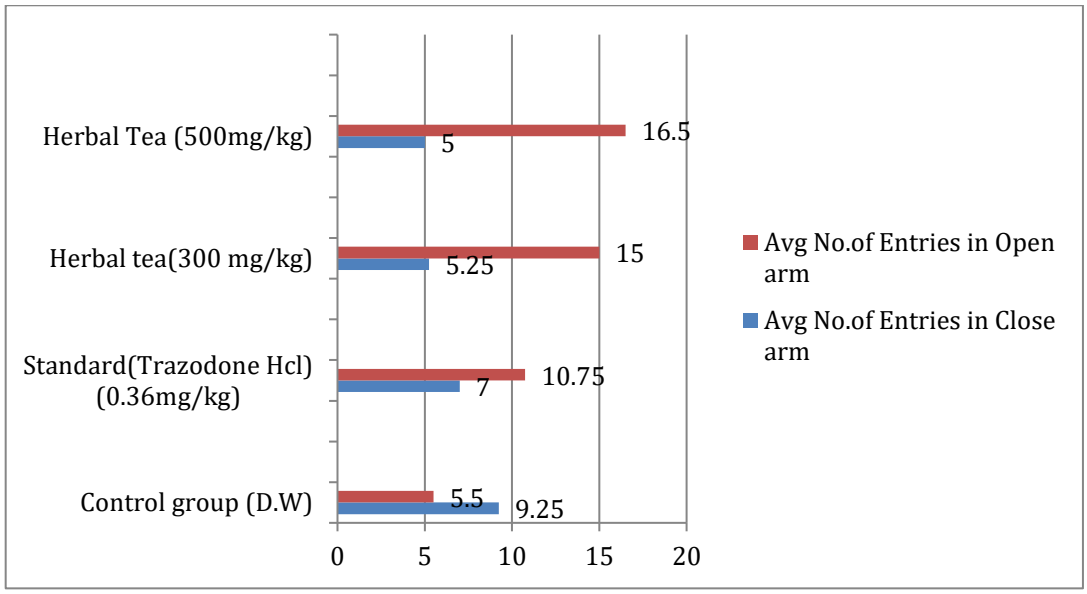


Figure 4: Average No. of Entries in Open and Close Arm of Elevated Plus Maze test

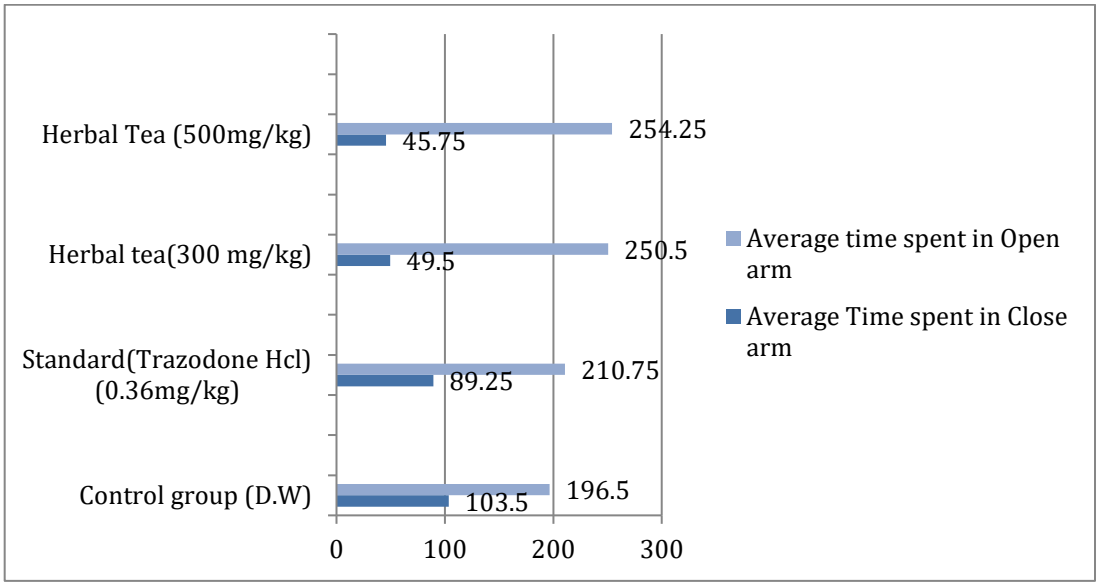


Figure 5: Average time spent in Open and Close arm of Elevated plus Maze test

4. DISCUSSION:

The study's findings show how the herbal components, including Passiflora incarnata leaves, Chamomile matricaria flowers, and Valerian officinalis root, were examined under a microscope. The diagnostic characteristics seen using powder microscopy reveal crucial details about the distinctive elements found in each plant material. Studies on Valerian officinalis' sedative and sleep-inducing qualities have been conducted. Linarin, a flavonoid derived from Valerian, was reported to have calming effects and to promote sleep in experimental models in one study(20). Additionally, valerian flavonoid glycosides' sedative effects have been documented (20). The historic usage of valerian as a natural sleep aid is supported by these findings. The microscopic analysis of the Valerian officinalis root showed the presence of reticulate xylem vessels, unicellular trichomes which represent the traces of stem epidermal hairs , cork cells which are secondary meristems formed in roots and stems after the tissues of the primary plant body have diferentiated, prismatic crystals of calcium oxalate (21, 22), phloem fibres are present as some roots possess primary phloem fibers , parenchymatous cells filled with starch grains basically they are present in cortex and pith of roots and stem, laticiferous vessels attached to the epidermal layer, polygonal cellulosic oil globules, aleurone grains, rosette crystal. The research by Cornara, L. et al., published in 2020, examined the valerian officinalis' microscopic characteristics to identify its sedative components. The article highlights the properties of Valeriana officinalis and details the microscopic analysis. The powdered root/rhizome of Valerian officinalis contains rectangular sclereids, xylem bundles, root hairs, and fragments of cork in addition to parenchyma fragments with starch grains and cells with resin. The root has a small central stele, a broad cortex with parenchyma cells loaded with starch, and a hypodermal layer with polygonal cells filled with oil. The rhizome exhibits starch-rich cortex, broad pith with parenchyma cells, and circularly organised vascular bundles. These tiny details shed light on the structural characteristics of Valeriana officinalis that are pertinent to its historical use as an antidepressant(23, 24, 25).

It has long been known that chamomile (Chamomile matricaria) offers calming and relaxing qualities. Its promise as a herbal medication for fostering relaxation and lowering anxiety has been suggested by several studies. According to one study, chamomile may have antidepressant effects on anxious and depressed people (25). The historical usage of chamomile as a natural remedy and its potential future medicinal uses were noted in another review (26). These studies offer scientific proof in favour of chamomile's historical use as a sedative and anxiety-relieving herb. Numerous research have been conducted to assess the microscopical characteristics of matricaria chamomilla. These studies offer scientific proof in favour of chamomile's historical use as a sedative and anxiety-relieving herb. Numerous research have been conducted to assess the microscopical characteristics of matricaria chamomilla. As In one study, it was discovered that there were sclereids in the middle of a bract or

palea, papillose stigma and a portion of the style, as well as outer epidermis close to the base of the corolla, all of which were connected with cluster crystals of calcium oxalate. The standardisation of the medication and the assessment of its potential pharmacological qualities depend on these histological traits, which provide useful insights into the composition and structure of Matricaria chamomilla flower powder(27).Multicellular trichomes, covering trichomes, scattered pollen grains, oil cells, forming trichomes on the epidermal layer, schizogenous oil glands, parenchyma, vascular tissues, lignified cork cells, cortical cells, groups of pollen grains, funiculus trichomes, and calcium oxalate crystals were all visible in the powder microscopy of Chamomile matricaria flowers. Others included columnar sclereids, cylindrical sclereids, septal fibres, unicellular trichomes, oil cells, groups of oil cells, sclereids, annular vascular elements, parenchyma tissues, parenchyma tissues with oil glands, and glandular trichomes. The identification and authentication of Chamomile blossoms are made easier with the aid of this microscopic analysis.

In the case of Passiflora incarnata leaves, the powder microscopy revealed the presence of trichomes, stomata, parenchymatous cells, square crystals of calcium oxalate, pollen grains, parts of the ovary represent the presence of traces of stamen, xylem vessels with spiral thickening, xylem vessels with reticulate thickening, unicellular trichomes, lignified fibers, capitate trichomes, thick-walled tracheids, groups of fibers, unicellular trichomes, epidermal cells, starch grains, prismatic crystals of calcium oxalate, groups of stomata, xylem vessels with spiral thickening, glangular cells, rectangular crystals of calcium oxalate, uniseriate trichomes, epidermal cells, fragments of epidermal cells, long trichomes, and vessels. S. Poonam conducted research. (2019) The leaf was dried, pulverised, and analysed under a scanning electron microscope in this study. The existence of unicellular twisted trichomes with glandular structures was identified by microscopic examination. The trichomes had a flat surface, but the epidermal wall cells had a wavy pattern. SEM microscopy offered extensive insights into the morphological aspects of Passiflora incarnata leaf structures(28). These minuscule characteristics help with Passiflora incarnata leaf identification and quality control. Passiflora incarnata (Passionflower) has been studied for its anxiolytic and sedative properties. Passionflower extracts have been demonstrated in studies to interact with the GABAergic system, which is implicated in anxiety management and sleep induction (29). Anxiolytic action of Passiflora incarnata aerial and subterranean portions was established in an experimental investigation (30). These findings imply that Passionflower has the potential to be used as a natural sedative and anti-anxiety drug.

Through the calculation of the lethal dose (LD50), the toxicity assessment of the herbal tea formulation was assessed. The findings showed that the herbal tea's LD50 was estimated to be 2400 mg/kg, indicating that it is safe to consume. Although there are few particular research on the toxicity of these herbs, generic references on the LD50 values of herbal extracts can shed light on their safety profiles. These sources offer instructions on determining the toxicity of herbal extracts, including "A Guide to the Handling of Genotoxic Carcinogens and Potent Compounds" by AstraZeneca R&D Safety Health and Environment (2001) and "Evaluation of Certain Food Additives and Contaminants" by the World Health Organisation (2011).

In preclinical and clinical research, the thiopental sodium-induced sleep test is employed to evaluate the sedative potential of numerous substances, including pharmaceutical medicines and herbal preparations. It aids in the understanding of the pharmacological characteristics of substances and how they affect sleep patterns, such as the onset, duration, and quality of sleep.

The findings showed that the herbal tea test group had a considerably quicker beginning of sleep than the control group ($p < 0.05$) when given doses of 300 mg/kg and 500 mg/kg of the tea. Additionally, compared to the control group, the test group's sleep duration at 500 mg/kg and 300 mg/kg was considerably longer ($p < 0.01$). These results imply that the herbal tea preparation has sleep-inducing properties. The results of Elevated plus maze test suggest that the herbal tea, particularly at 300 mg/kg and 500 mg/kg doses, effectively reduced anxiety and promoted exploratory behavior in the EPM test, making it a potential candidate for anxiety management. The study's findings are intriguing, but they are not without limits. Because the mouse model employed may not be directly applicable to humans, human clinical trials are required for confirmation. Variability in herbal content owing to growth conditions must be taken into account when scaling for human use. While microscopic examination

revealed insights, it did not provide a complete chemical profile, necessitating additional chemical investigations. Finally, while the LD50 in mice implies safety, human safety, particularly with long-term usage, requires further examination. Several critical topics require attention in the future. To begin, clinical trials are required to validate the herbal tea's efficacy and safety in humans, allowing for dosage determination and side effect assessment. In-depth chemical analysis is also required to determine the specific sedative chemicals, ensuring product standardisation and quality control. Optimising the formulation, maybe by altering herbal ratios or investigating supplementary herbs, could increase its effectiveness while maintaining its safety. Exploring clinical applications outside of insomnia, particularly in anxiety management, can help to increase its therapeutic potential. Finally, while this study provides promising findings, addressing these difficulties will pave the road for safe and effective herbal therapies for sleep and anxiety issues.

5. CONCLUSION:

Overall, the comprehensive analysis of herbal components and the substantiated therapeutic effects of the herbal tea formulation suggest its potential as a natural remedy for insomnia and anxiety-related conditions, warranting further exploration in clinical applications and can be used as a life style medicine for healthy sleep pattern.

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REFERENCES:

1. Dey A. Phytotherapy against insomnia: extravagant claims or an alternative medicine? Pakistan Journal of Biological Sciences: PJBS. 2013;16(3):148-50. URL https://pubmed.ncbi.nlm.nih.gov/24171278/
2. Orzeł-Gryglewska J. Consequences of sleep deprivation. Int J Occup Med Environ Health. 2010;23(1):95-114. https://pubmed.ncbi.nlm.nih.gov/20442067/
3. Zaharna M, Guilleminault C. Sleep, noise and health: review. Noise Health. 2010;12(47):64-9. https://www.noiseandhealth.org/article.asp?issn=1463-1741; year=2010; volume=12; issue=47; spage=64; epage=69; aulast=Zaharna
4. McPherson F, McGraw L. Treating generalized anxiety disorder using complementary and alternative medicine. Altern Ther Health Med. 2013;19(5):45-50. https://bmccomplementmedtherapies.biomedcentral.com/articles/10.1186/1472-6882-12-S1-P130#citeas
5. Cho SM, Shimizu M, Lee CJ, et al. Hypnotic effects and binding studies for GABA(A) and 5-HT(2C) receptors of traditional medicinal plants used in Asia for insomnia. J Ethnopharmacol. 2010;132(1):225-32. https://pubmed.ncbi.nlm.nih.gov/20804838/
6. Roth T, Drake C. Evolution of insomnia: current status and future direction. Sleep Med. 2004;5 Suppl 1:S23-30. https://pubmed.ncbi.nlm.nih.gov/15301994/
7. Phillipson JD. Phytochemistry and medicinal plants. Phytochemistry. 2001;56(3):237-43. https://pubmed.ncbi.nlm.nih.gov/11243450/
8. Carlini EA. Plants and the central nervous system. Pharmacol Biochem Behav. 2003;75(3):501-12. https://pubmed.ncbi.nlm.nih.gov/12895668/
9. Zhang X, Lu Y, Lv F, et al. Valerian for Insomnia on Subjective and Objective Sleep Parameters: a Meta-analysis of Randomized Controlled Trials. Current Sleep Medicine Reports. 2023:1-14. https://www.researchgate.net/publication/371638474_Valerian_for_Insomnia_on_Subjective_and_Objective_Sleep_Parameters_a_Meta-analysis_of_Randomized_Controlled_Trials

10. Wang W, Wang Y, Guo Q, et al. Valerian essential oil for treating insomnia via the serotonergic synapse pathway. Frontiers in Nutrition. 2022;9:927434. https://www.frontiersin.org/articles/10.3389/fnut.2022.927434/full
11. Adib-Hajbaghery M, Mousavi SN. The effects of chamomile extract on sleep quality among elderly people: A clinical trial. Complementary therapies in medicine. 2017;35:109-14. https://pubmed.ncbi.nlm.nih.gov/29154054/
12. Kim GH, Kim Y, Yoon S,et al. Sleep-inducing effect of Passiflora incarnata L. extract by single and repeated oral administration in rodent animals. Food science & nutrition. 2020;8(1):557-66. https://pubmed.ncbi.nlm.nih.gov/31993179/
13. Akhila JS, Shyamjith D, Alwar M. Acute toxicity studies and determination of median lethal dose. Current science. 2007:917-20. https://currentscience.ac.in/Volumes/93/07/0917.pdf
14. Askari VR, Baradaran Rahimi V, Ghorbani A et al. Hypnotic Effect of Ocimum basilicum on Pentobarbital-Induced Sleep in Mice. Iran Red Crescent Med J. 2016;18(7):e24261. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5020426/
15. Hosseini A, Forouzanfar F, Rakhshandeh H. Hypnotic effect of Nepeta glomerulosa on pentobarbital-induced sleep in mice. Jundishapur Journal of Natural Pharmaceutical Products. 2016;11(1). https://brieflands.com/articles/jjnpp-18428.html
16. Oliaee D, Boroushaki MT, Oliaee N, Ghorbani A. Evaluation of cytotoxicity and antifertility effect of Artemisia kopetdaghensis. Advances in Pharmacological and Pharmaceutical Sciences. 2014;2014. https://pubmed.ncbi.nlm.nih.gov/24711816/
17. Moniruzzaman M, Rahman A, Ferdous A. Evaluation of sedative and hypnotic activity of ethanolic extract of Scoparia dulcis Linn. Evidence-Based Complementary and Alternative Medicine. 2015;2015. https://www.hindawi.com/journals/ecam/2015/873954/
18. Moniruzzaman M, Sharoti Bhattacharjee P, Rahman Pretty M, Sarwar Hossain M. Sedative and Anxiolytic-Like Actions of Ethanol Extract of Leaves of Glinus oppositifolius (Linn.) Aug. DC. Evid Based Complement Alternat Med. 2016;2016:8541017. https://pubmed.ncbi.nlm.nih.gov/27413390/
19. de Figueiredo Cerqueira MM, Castro MML, Vieira AA, Kurosawa JAA, do Amaral Junior FL, de Siqueira FdCC, et al. Comparative analysis between Open Field and Elevated Plus Maze tests as a method for evaluating anxiety-like behavior in mice. Heliyon. 2023;9(4). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10070366/
20. Fernández S, Wasowski C, Paladini AC, Marder M. Sedative and sleep-enhancing properties of linarin, a flavonoid-isolated from Valeriana officinalis. Pharmacology Biochemistry and Behavior. 2004;77(2):399-404. https://pubmed.ncbi.nlm.nih.gov/14751470/
21. Sutherland J, Sprent J. Calcium-oxalate crystals and crystal cells in determinate root nodules of legumes. Planta. 1984;161:193-200. https://www.jstor.org/stable/23377463
22. Tütüncü Konyar S, Öztürk N, Dane F. Occurrence, types and distribution of calcium oxalate crystals in leaves and stems of some species of poisonous plants. Botanical studies. 2014;55(1):1-9. https://as-botanicalstudies.springeropen.com/articles/10.1186/1999-3110-55-32
23. Cornara L, Ambu G, Trombetta D et al. Comparative and functional screening of three species traditionally used as antidepressants: Valeriana officinalis L., valeriana jatamansi jones ex roxb. and nardostachys jatamansi (D. Don) DC. Plants. 2020;9(8):994. https://pubmed.ncbi.nlm.nih.gov/32764268/
24. Houghton P. Valerian: the genus Valeriana: Crc Press; 1997.
25. Upton R, Graff A, Jolliffe G, Länger R, Williamson E. American herbal pharmacopoeia: botanical pharmacognosy-microscopic characterization of botanical medicines: CRC Press; 2016.
26. Srivastava JK, Shankar E, Gupta S. Chamomile: A herbal medicine of the past with a bright future. Molecular medicine reports. 2010;3(6):895-901. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2995283/
27. Khan N, Kalam MA, Alam MT, et al. Drug Standardization through Pharmacognostic Approaches and Estimation of Anticancer Potential of Chamomile (Matricaria chamomilla L.)

using Prostate-Cancer cell lines: An In-vitro Study. Journal of Cancer. 2023;14(3):490. https://www.jcancer.org/v14p0490.htm
28. Sethi P. SCANNING ELECTRON MICROSCOPIC ANALYSIS OF PASSIFLORA INCARNATA LINN LEAF. Chemistry & Material Sciences Research Journal. 2020;2(1):1-5. https://actascientific.com/ASAG/pdf/ASAG-03-0701.pdf
29. Guerrero FA, Medina GM. Effect of a medicinal plant (Passiflora incarnata L) on sleep. Sleep Sci. 2017;10(3):96-100. https://pubmed.ncbi.nlm.nih.gov/29410738/
30. Dhawan K, Kumar S, Sharma A. Anxiolytic activity of aerial and underground parts of Passiflora incarnata. Fitoterapia. 2001;72(8):922-6. https://pubmed.ncbi.nlm.nih.gov/11731118/

LIST OF ABBREVIATION:

- PI - Passiflora incarnata L.
- LD50 - Lethal Dose 50
- HCl - Hydrochloride
- SPSS - Statistical Package for the Social Sciences
- SEM - Scanning Electron Microscope
- GABA - Gamma-Aminobutyric Acid
- EPM - Elevated Plus Maze
- PCSIR - Pakistan Council of Scientific and Industrial Research
- NREM - Non-Rapid Eye Movement
- ATP - Adenosine Triphosphate
- 5-HT1AR - 5-Hydroxytryptamine Receptor 1A
- cAMP - Cyclic Adenosine Monophosphate
- PKA - Protein Kinase A
- EPM - Elevated Plus Maze
- ANOVA - Analysis of Variance