Phytochemical Investigation and Pharmacological Evaluation of Butea monosperma root Extract for Antianxiety Activity in Rats

Mr. Pratik Laxman Kolte [1], Dr. Karna B. More[2]
Student[1], Research Guide [2]
[1,2] Dr. Vedprakash Patil Pharmacy College

Abstract- The present study was undertaken to investigate the phytochemical constituents and pharmacological potential of *Butea monosperma* root extract with special emphasis on its antioxidant and antianxiety activities in experimental animals. The plant material was collected, authenticated, dried, and subjected to pharmacogenetic and physicochemical evaluation to confirm its identity and quality. The powdered root material was extracted using different solvents, and the obtained extracts were evaluated for their phytochemical profile and biological activities.

Preliminary phytochemical investigation revealed the presence of various bioactive constituents including alkaloids, flavonoids, glycosides, tannins, phenolic compounds, proteins, and carbohydrates. Quantitative estimation showed the presence of significant amounts of phenolic and flavonoid compounds, indicating the potential antioxidant capacity of *Butea monosperma* root extracts.

The antioxidant activity was evaluated using the DPPH free radical scavenging method. The extracts demonstrated significant concentration-dependent free radical scavenging activity, suggesting their ability to reduce oxidative stress. The ethanolic extract showed superior antioxidant potential due to the higher content of phenolic and flavonoid constituents.

The pharmacological evaluation of antianxiety activity was carried out using the Elevated Plus Maze (EPM) model in Wistar rats. Treatment with ethanolic and methanolic extracts of *Butea monosperma* root showed a significant increase in open arm entries and time spent in open arms, along with a reduction in closed arm preference, indicating anxiolytic activity. The observed effect was comparable with the standard anxiolytic drug diazepam.

Acute toxicity studies revealed that the extracts were safe and well tolerated at the tested dose levels. The antianxiety activity of *Butea monosperma* root extract may be attributed to the presence of phenolic compounds, flavonoids, and other secondary metabolites responsible for neuroprotective and antioxidant effects.

The findings of the present study provide scientific evidence supporting the traditional use of *Butea monosperma* and suggest its potential as a natural therapeutic agent for the management of anxiety disorders. Further investigations are required for isolation of active phytoconstituents and molecular studies to establish its mechanism of action.

Keyword: Butea monosperma root extract, phytochemical investigation, antianxiety activity, antioxidant activity, Elevated Plus Maze, Wistar rats, diazepam.

I. INTRODUCTION

Anxiety is a normal emotional response that occurs in reaction to stressful or threatening situations and plays an important role in survival by preparing the body to respond to danger. However, when anxiety becomes excessive, persistent, and disproportionate to the actual situation, it develops into a pathological condition known as an anxiety disorder. Anxiety disorders are among the most prevalent mental health disorders worldwide and affect individuals across different age groups. These disorders are associated with excessive fear, continuous worry, avoidance behavior, restlessness, and disturbances in cognitive and physiological functions.

Anxiety disorders include several clinical conditions such as generalized anxiety disorder (GAD), panic disorder, social anxiety disorder, specific phobia, separation anxiety disorder, and agoraphobia. These disorders significantly influence emotional stability,

academic performance, occupational productivity, and social interactions. Due to their chronic nature and high recurrence rate, anxiety disorders contribute considerably to reduced quality of life and increased healthcare burden.

The pathophysiology of anxiety is complex and involves multiple factors including genetic predisposition, environmental stress, neurochemical imbalance, and alterations in brain circuitry. The amygdala, hippocampus, and prefrontal cortex are important brain regions involved in fear and anxiety regulation. Abnormal activation of the amygdala and impaired regulation by the prefrontal cortex contribute to exaggerated fear responses and anxiety symptoms.

Neurotransmitters such as serotonin (5-hydroxytryptamine), gamma-aminobutyric acid (GABA), dopamine, and norepinephrine play essential roles in maintaining emotional balance. Reduced serotonergic activity and altered GABAergic transmission have been associated with anxiety-related behaviors. The hypothalamic–pituitary–adrenal (HPA) axis also plays an important role in stress response, and prolonged activation of this pathway may contribute to anxiety development.

Currently available treatments for anxiety disorders include psychotherapy and pharmacological interventions. Cognitive behavioral therapy and other psychological approaches are effective in reducing anxiety symptoms. Pharmacological treatments mainly involve antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs). Benzodiazepines are also widely used due to their rapid anxiolytic action; however, their long-term administration may cause tolerance, dependence, sedation, and withdrawal effects.

The limitations associated with synthetic anxiolytic drugs have increased interest in medicinal plants as alternative therapeutic agents. Herbal medicines contain diverse phytoconstituents that may act through multiple mechanisms, including antioxidant activity, modulation of neurotransmitter systems, and neuroprotection. Natural compounds such as flavonoids, polyphenols, alkaloids, and terpenoids have been reported to possess significant effects on the central nervous system.

Oxidative stress has gained attention as an important factor involved in neurological and psychiatric disorders. Excessive generation of reactive oxygen species (ROS) can damage neuronal cells, alter neurotransmitter balance, and affect brain function. Antioxidant phytochemicals may protect neurons by neutralizing free radicals and maintaining cellular integrity. Therefore, plants rich in phenolic and flavonoid compounds are considered promising candidates for investigation in anxiety-related disorders.

Butea monosperma (Lam.) Kuntze, belonging to the family Fabaceae, is a well-known medicinal plant commonly called “Palash” or “Flame of the Forest.” It is widely distributed in India, Sri Lanka, Myanmar, and other tropical regions. Traditionally, different parts of *Butea monosperma* have been used in Ayurveda and folk medicine for various therapeutic purposes.

The roots of *Butea monosperma* are traditionally used for several medicinal applications and contain a wide range of secondary metabolites responsible for pharmacological activities. Phytochemical investigations of *Butea monosperma* have reported the presence of flavonoids, tannins, phenolic compounds, glycosides, alkaloids, and other bioactive molecules. These constituents are associated with antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and neuroprotective activities.

Flavonoids present in medicinal plants have attracted considerable attention due to their ability to influence neuronal signaling pathways. They may interact with GABA receptors, reduce oxidative damage, and regulate inflammatory processes involved in anxiety. Phenolic compounds also contribute to antioxidant defense mechanisms by scavenging free radicals and preventing oxidative injury.

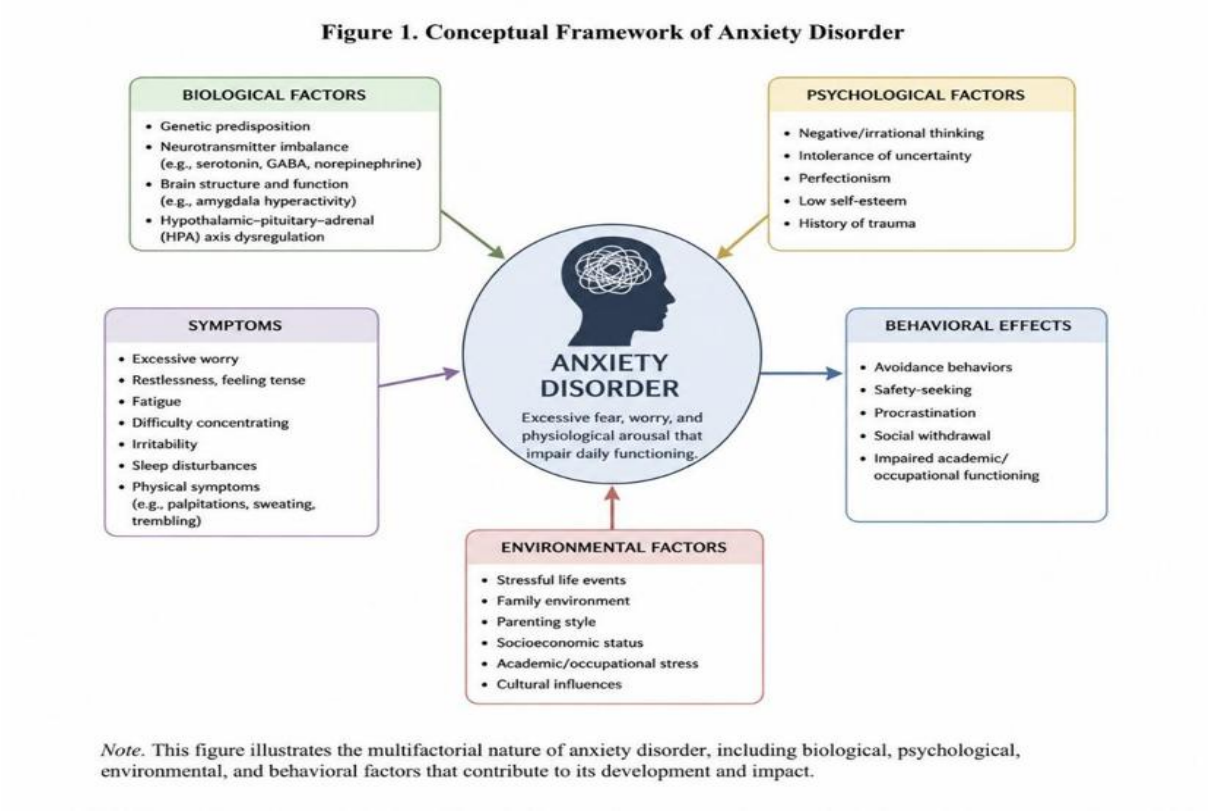


Fig.01 Conceptual Framework of Anxiety Disorder

Experimental animal models are essential tools for evaluating the pharmacological activity of potential anxiolytic agents. Among various behavioral models, the Elevated Plus Maze (EPM) model is widely accepted for screening anti-anxiety activity. The model is based on the natural conflict between the animal's desire to explore and its fear of open elevated spaces. Compounds possessing anxiolytic activity increase exploratory behavior, reflected by increased open arm entries and time spent in open arms.

The selection of *Butea monosperma* root extract for the present investigation is based on its traditional medicinal importance and reported presence of biologically active compounds. However, scientific validation of its antianxiety potential requires systematic evaluation through pharmacological studies.

Therefore, the present study was undertaken with the objective of phytochemical investigation and pharmacological evaluation of *Butea monosperma* root extract for antianxiety activity in rats. The study aims to evaluate the phytochemical profile, antioxidant potential, and behavioral effects of root extracts using experimental models.

The results obtained from this investigation may contribute to understanding the therapeutic potential of *Butea monosperma* as a natural source for the development of safer and effective anxiolytic agents.

Medicinal plants have been considered an important source of therapeutic agents since ancient times. Traditional systems of medicine such as Ayurveda, Siddha, and Unani have documented the use of various plants for the management of neurological and psychological disorders. The increasing interest in herbal medicines is mainly due to their broad pharmacological activities, lower incidence of adverse effects, and presence of multiple active constituents that may act synergistically.

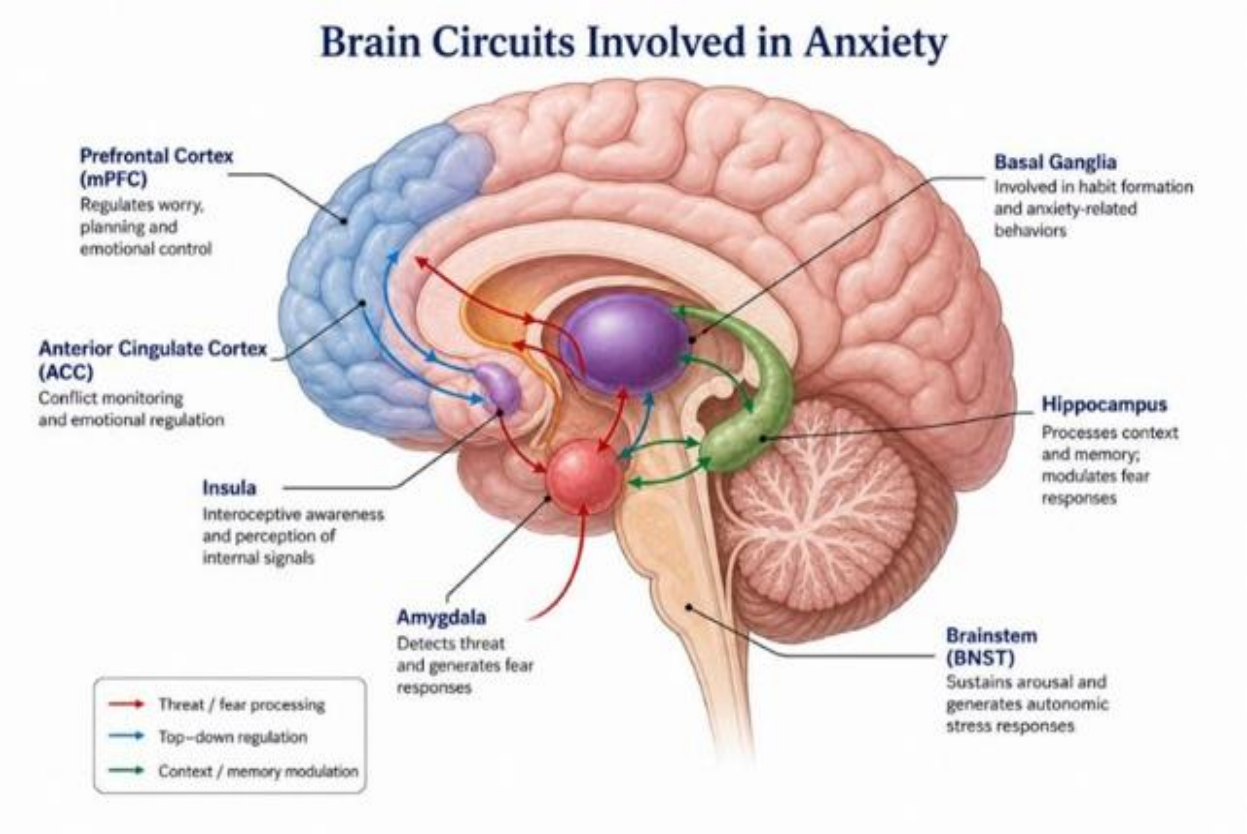


Fig 02 Anxiety Brain.

The evaluation of medicinal plants for central nervous system (CNS) activity has gained importance due to the growing prevalence of stress-related disorders. Several plant-derived compounds have demonstrated anxiolytic properties by affecting neurotransmitter pathways involved in anxiety regulation. Flavonoids and phenolic compounds have been particularly studied for their ability to interact with benzodiazepine receptors, enhance GABAergic transmission, and reduce neuronal excitability.

Butea monosperma is one such medicinal plant with a long history of traditional use. The plant is commonly known as “Dhak,” “Palash,” or “Flame of the Forest” and is considered an important medicinal tree in Indian traditional medicine. Various parts of the plant have been reported to possess pharmacological properties, including antioxidant, anti-inflammatory, analgesic, antimicrobial, antidiabetic, and hepatoprotective effects.

The root of *Butea monosperma* contains several phytoconstituents that may contribute to its biological activity. Phytochemical components such as flavonoids, phenolic compounds, tannins, and glycosides have been associated with protective effects on the nervous system. These compounds may help in maintaining neuronal function by reducing oxidative stress and modulating pathways involved in emotional regulation.

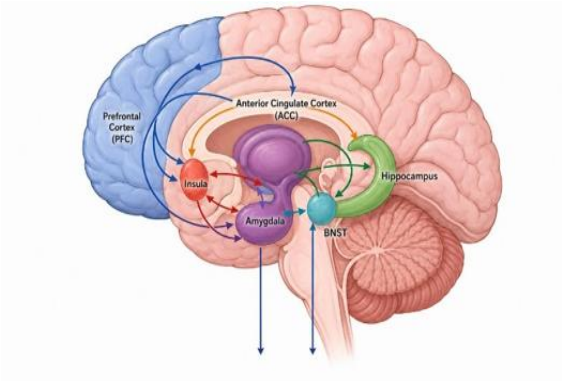


Fig.03.Neurobiology of Anxiety

Oxidative stress occurs due to an imbalance between the production of reactive oxygen species and the

antioxidant defense system of the body. Excessive oxidative stress may affect brain function by damaging neuronal membranes, altering neurotransmitter metabolism, and influencing synaptic communication. Since the brain has high oxygen consumption and contains abundant lipid content, it is particularly vulnerable to oxidative damage. Therefore, antioxidant-rich plant extracts may provide beneficial effects in neurological disorders, including anxiety.

The relationship between oxidative stress and anxiety-like behavior has been investigated in several experimental studies. Increased levels of oxidative markers and reduced antioxidant defense have been reported in stress-induced animal models. Therefore, plants possessing strong antioxidant activity may have potential in reducing anxiety-related behavioral change

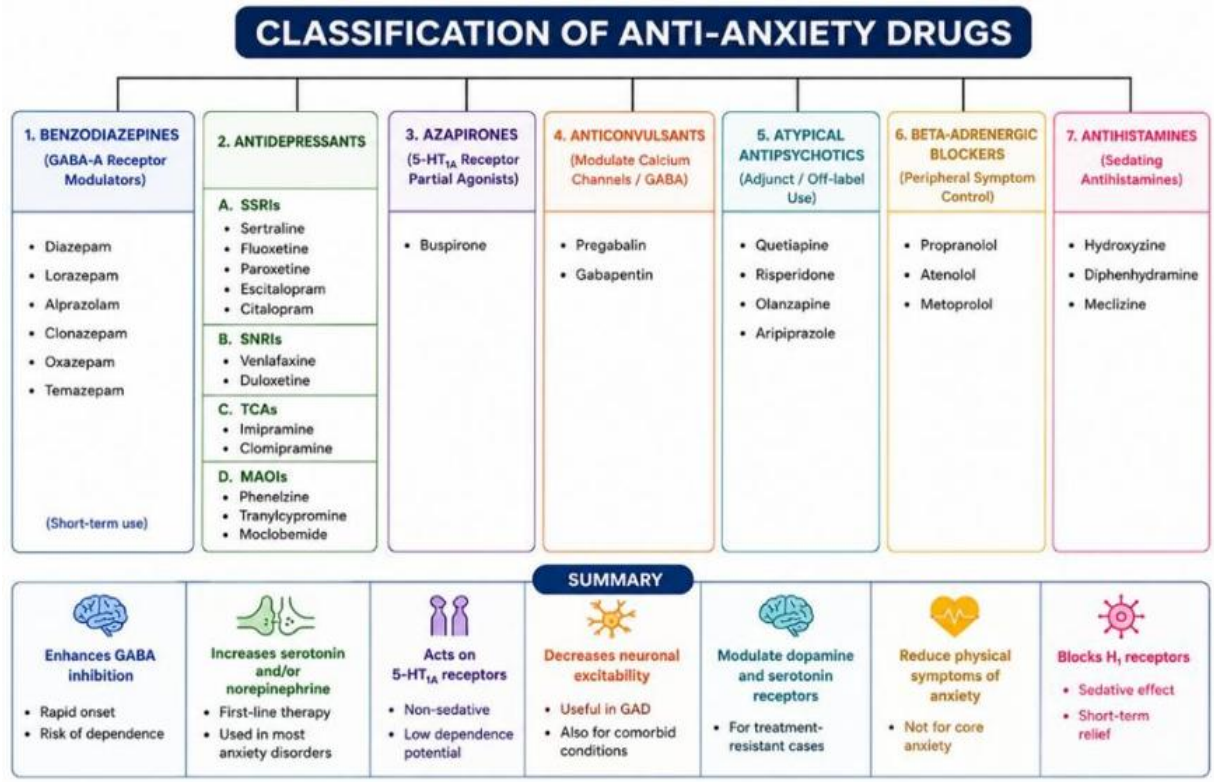


Fig.04 Drugs Classification

The pharmacological screening of plant extracts requires appropriate experimental models to evaluate their biological effects. Animal models provide valuable information regarding

behavioral changes and possible mechanisms of action. The Elevated Plus Maze (EPM) test is one of the most commonly used and validated models for assessing anxiolytic activity in rodents.

The EPM model consists of two open arms and two closed arms elevated above the ground. Rodents naturally avoid open and elevated areas due to fear, but anxiolytic agents increase their exploratory behavior. The parameters such as number of entries into open arms and time spent in open arms are considered important indicators of reduced anxiety-like behavior.

In addition to behavioral studies, preliminary phytochemical evaluation provides important information regarding the chemical composition of plant extracts. Screening for alkaloids, flavonoids, glycosides, tannins, phenolics, and other constituents helps in identifying possible compounds responsible for pharmacological effects.

Quantitative estimation of phenolic and flavonoid contents is also essential because these compounds are strongly correlated with antioxidant activity. Higher phenolic and flavonoid levels generally indicate

greater free radical scavenging capacity and potential therapeutic effectiveness.

AIM:

"Phytochemical Investigation and Pharmacological Evaluation of *Butea monosperma* Root Extract for Anti-anxiety Activity in Rats."

OBJECTIVES:

1. To carry out the phytochemical investigation and standardization of *Butea monosperma* root extracts.
2. To evaluate the in-vitro antioxidant potential of *Butea monosperma* root extracts using suitable antioxidant assays.
3. To perform qualitative and quantitative phytochemical screening of *Butea monosperma* root extracts for identification of bioactive constituents.
4. To conduct preclinical pharmacological evaluation of *Butea monosperma* root extracts for anti-anxiety activity using suitable animal models.

II.LITERATURE REVIEW

Butea monosperma (Lam.) Kuntze, commonly known as "Palas", "Dhak", or "Flame of the Forest", belongs to the family Fabaceae. It is a medium-sized deciduous tree widely distributed throughout India, Sri Lanka, and surrounding regions. The plant has significant medicinal importance and has been traditionally used in Ayurveda for various disorders. Different parts of Butea monosperma contain several bioactive constituents responsible for its pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, hypoglycemic, and hepatoprotective effects. The plant is rich in phytoconstituents including flavonoids, glycosides, tannins, and terpenoids, which contribute to its therapeutic potential. Due to its extensive traditional applications, Butea monosperma has gained considerable attention for scientific evaluation and development of plant-based therapeutic agents.

Medicinal plants serve as an important source of natural bioactive compounds and have been used in healthcare systems since ancient times. Herbal medicines are preferred due to their availability, affordability, and relatively lower adverse effects compared with synthetic drugs. Various plant parts such as roots, bark, leaves, flowers, and seeds possess medicinal value. Butea monosperma is an important Ayurvedic medicinal plant traditionally used for the management of gastrointestinal disorders, inflammation, infections, liver disorders, and metabolic conditions. The presence of diverse phytochemicals makes this plant a promising candidate for pharmacological investigations and drug development.

Bioactive compounds derived from medicinal plants have gained importance due to their antioxidant and therapeutic properties. Butea monosperma contains several secondary metabolites that contribute to its biological activities. Studies on different extracts of Butea monosperma have demonstrated significant antioxidant potential associated with phenolic and flavonoid compounds. Phytochemical analysis revealed the presence of various active constituents responsible for reducing oxidative stress and inflammatory responses. These findings indicate the potential application of Butea monosperma extracts in disorders associated with oxidative imbalance.

Peptic ulcer is a chronic gastrointestinal disorder associated with damage to gastric mucosa. Although synthetic drugs are effective, their long-term use may cause adverse effects. Therefore, herbal medicines have gained attention as alternative therapeutic approaches. Several medicinal plants containing flavonoids, tannins, and phenolic compounds exhibit protective effects through antioxidant and anti-inflammatory mechanisms. Such phytoconstituents present in medicinal plants including Butea monosperma may contribute to therapeutic effects against various pathological conditions.

Butea monosperma has been traditionally used in Indian medicine for treating various disorders. Different parts of the plant including roots, bark, leaves, flowers, and seeds possess medicinal value. The plant exhibits pharmacological activities such as anti-inflammatory, antioxidant, antimicrobial, and hepatoprotective effects. Pharmacognostic and

physicochemical evaluations of Butea monosperma are important for identification, quality control, and standardization of herbal drugs.

Butea monosperma flowers contain important phytoconstituents such as butein, butrin, and isobutrin, which possess antioxidant properties. These compounds have demonstrated protective effects against oxidative stress-mediated cellular damage. The antioxidant activity of Butea monosperma suggests its possible role in managing disorders associated with oxidative stress and inflammation.

Butea monosperma, known as “Flame of the Forest”, is a medicinally important tree belonging to Fabaceae family. Traditionally, different parts of the plant are used in Ayurveda, Unani, and folk medicine. Phytochemical studies have reported the presence of flavonoids, terpenoids, steroids, and glycosides responsible for its antioxidant, anti-inflammatory, antimicrobial, and antidiabetic activities.

Medicinal plants are valuable sources of antimicrobial and therapeutic compounds. Extracts of Butea monosperma have demonstrated antibacterial activity against various pathogenic microorganisms. Phytochemical screening confirmed the presence of bioactive compounds responsible for inhibition of microbial growth. These findings support the importance of Butea monosperma as a potential source of natural therapeutic agents.

The increasing side effects associated with synthetic drugs have enhanced interest in herbal medicines. Butea monosperma has been extensively used traditionally for the treatment of fever, diabetes, inflammation, infections, and gastrointestinal disorders. The presence of flavonoids, diterpenoids, lactones, and phytosterols contributes to its pharmacological activities and therapeutic importance.

Butea monosperma possesses significant ethnomedicinal and pharmacological importance. Scientific studies have reported its antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, and anticancer activities. The therapeutic properties of this plant are mainly attributed to flavonoids, glycosides, diterpenoids, and other bioactive compounds.

Butea monosperma belongs to the Fabaceae family and is widely distributed in tropical and subtropical regions. It is traditionally used for treating various disorders such as inflammation, liver diseases, infections, and neurological conditions. Phytochemical studies have reported the presence of flavonoids, chalcones, triterpenoids, steroids, and glycosides. The presence of these bioactive constituents supports its pharmacological significance and potential application in herbal drug development.

Butea monosperma has been used in traditional medicine due to its wide range of therapeutic properties. The plant exhibits antimicrobial, wound healing, antifungal, antioxidant, anti-inflammatory, hepatoprotective, antidiabetic, and anticonvulsant activities. The pharmacological effects are mainly associated with phytoconstituents such as butein, butrin, isobutrin, and palasitrin.

The formulation and evaluation of herbal preparations using Butea monosperma have gained attention due to its medicinal properties. The flowers of Butea monosperma possess antioxidant, antidiabetic, anti-stress, hepatoprotective, and antimicrobial activities. Herbal formulations prepared from standardized plant materials are evaluated for quality parameters to ensure safety, stability, and therapeutic effectiveness.

Butea monosperma has been traditionally used in various indigenous systems of medicine due to its rich phytochemical composition and pharmacological activities. Review studies have reported the presence

of flavonoids, diterpenoids, lactones, phytosterols, and glycosides. The plant exhibits antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer, and hepatoprotective activities.

Butea monosperma is widely recognized in Ayurveda and traditional medicine for its therapeutic applications. Preliminary phytochemical studies of different extracts have revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, steroids, and terpenoids. These

phytoconstituents contribute to its antioxidant potential and medicinal properties.

Medicinal plants containing bioactive compounds have been explored for their anxiolytic potential. Extracts of medicinal plants have demonstrated anti-anxiety activity in experimental animal models such as the Elevated Plus Maze. The anxiolytic effects are mainly attributed to phytochemicals that influence neurotransmitter systems, particularly the GABAergic pathway.

Oxidative stress plays an important role in neurological disorders including anxiety. Plants rich in phenolic compounds and flavonoids exhibit antioxidant properties that may help in reducing anxiety-related responses. Studies on medicinal plant extracts have demonstrated significant anxiolytic activity in animal models through behavioral tests.

Anxiety disorders are common neurological conditions characterized by emotional and behavioral disturbances. Conventional anxiolytic drugs mainly act through modulation of neurotransmitter systems such as GABA. Several medicinal plants containing flavonoids, alkaloids, tannins, and terpenoids have shown promising anti-anxiety activity by regulating neurotransmitter pathways.

Butea monosperma (Lam.) Kuntze, also known as Butea frondosa, belongs to the family Fabaceae and is a medium-sized deciduous tree widely distributed in India, Burma, and Sri Lanka. It is commonly called “Dhak” or “Palas” and is popularly known as the “Flame of the Forest” due to its attractive orange-red flowers. The plant has been extensively used in traditional systems of medicine because of its various therapeutic properties. Extracts obtained from different parts of Butea monosperma and its isolated phytoconstituents have demonstrated several biological activities including antibacterial, antifungal, hypoglycemic, antioxidant, and anti-inflammatory effects. Traditionally, the plant is considered to possess tonic, astringent, aphrodisiac, and diuretic properties. Due to its medicinal importance, extensive phytochemical investigations have been carried out to identify its active constituents and explore its pharmacological potential.

4. PLAN OF WORK

Collection, Identification and Authentication of Plant Material
• Collection of selected plant material (*Butea monosperma* root).
• Identification and authentication of the plant material by a qualified botanist/taxonomist.

Pharmacogenetic Evaluation of Plant Material
• Macroscopic and microscopic evaluation of plant material.
• Determination of physicochemical parameters for standardization of crude drug.

Processing of Crude Drug
• Cleaning, shade drying, pulverization, and preparation of powdered plant material.
• Storage of processed crude drug under suitable conditions for further studies.

Extraction of Plant Material
• Selection of suitable extraction method.
• Selection of appropriate solvent for extraction.
• Preparation and collection of plant extract.

Phytochemical Screening and Standardization of Extract

Physicochemical Evaluation:
• Determination of extractive values.
• Determination of total ash content.
• Determination of acid-insoluble ash and water-soluble ash.
• Determination of loss on drying/moisture content.

Qualitative Phytochemical Analysis:
The prepared extract will be screened for the presence of various phytoconstituents such as:
• Carbohydrates
• Alkaloids
• Tannins
• Flavonoids
• Terpenoids
• Lipids
• Proteins
• Phenolic compounds
• Other secondary metabolites

Quantitative Phytochemical Analysis:
• Estimation of total phenolic content.
• Estimation of total flavonoid content.

Pharmacological Evaluation of Plant Extract

In-vitro Studies:
• Evaluation of antioxidant activity of extract.

In-vivo Pharmacological Studies:
• Evaluation of anti-anxiety activity of *Butea monosperma* root extract using suitable animal models.

Data Analysis and Presentation
• Statistical analysis and interpretation of experimental results.
• Compilation and presentation of obtained data.

DRUG PROFILE:
Butea monosperma (Lam.) Taub.

Medicinal plants are considered important sources of biologically active compounds and have contributed significantly to the development of traditional and modern medicines. The increasing interest in herbal drugs is mainly due to their diverse pharmacological activities and presence of naturally occurring phytoconstituents.

Butea monosperma (Lam.) Taub. is an important medicinal plant belonging to the family Fabaceae. It is commonly known as Palash, Dhak, and Flame of the Forest. The plant is widely distributed in India and other parts of South Asia and has been traditionally used in Ayurveda, Unani, and folk medicine.

Different parts of Butea monosperma including flowers, leaves, seeds, bark, gum, and roots possess significant medicinal properties. The plant contains various phytoconstituents such as flavonoids, phenolic compounds, tannins, triterpenoids, glycosides, and steroids. Major reported compounds include butein, butin, monospermoside, isomonospermoside, medicarpin, lupeol, and β-sitosterol.

Research studies have demonstrated that Butea monosperma exhibits various pharmacological activities including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anticancer, wound healing, anticonvulsant, hepatoprotective, and neuroprotective activities.

The antioxidant activity of the plant is mainly attributed to flavonoids and phenolic compounds, which help in scavenging free radicals and protecting cells from oxidative damage. The anti-inflammatory activity is associated with inhibition of inflammatory mediators and regulation of inflammatory pathways.

Experimental studies have reported the antimicrobial potential of Butea monosperma extracts against several bacterial and fungal strains. The plant extracts have also shown significant hypoglycemic activity by reducing blood glucose levels and improving antioxidant defense mechanisms.

III.MATERIALS AND METHODS

MATERIAL

All chemicals and reagents used in the study were of analytical grade and obtained from standard suppliers. Solvents such as petroleum ether, ethanol, methanol, chloroform, acetone and ethyl acetate were used for extraction and analysis.

Major instruments used were analytical balance, Soxhlet apparatus, UV-visible spectrophotometer, centrifuge, desiccator, microscope, sonicator and hot air oven.

Sr.no	Drugs and Chemicals	Manufacturer
1	Petroleum Ether	Fine Chem Industries, Mumbai
2	Chloroform	Fine Chem Industries, Mumbai
3	Methanol	Fine Chem Industries, Mumbai
4	Ethanol	Fine Chem Industries, Mumbai

5	Benzene	Fine Chem Industries, Mumbai
6	Ethyl Acetate	Fine Chem Industries, Mumbai
7	Sulphuric Acid	Hi Media Lab. Mumbai
8	Pholoroglucinol	Loba Chemie Pvt. Ltd. Mumbai
9	DPPH	Hi Media Lab. Mumbai
10	Ascorbic Acid	Hi Media Lab. Mumbai
11	Hytrogen peroxide	Hi Media Lab. Mumbai
12	Gallic Acid	Loba Chemie Pvt. Ltd. Mumbai
13	Indomethacin	Loba Chemie Pvt. Ltd. Mumbai
14	Hydrochloride Acid	Loba Chemie Pvt. Ltd. Mumbai
15	Silica gel	Hi Media Lab. Mumbai
16	Ferric chloride	Research Lab. Fine Chem. Industries, Mumbai
17	Diazepam	Advacare Pharma
18	Sodium hydroxide	Loba Chemie Pvt. Ltd. Mumbai
19	Acetone	Fine Chem Industries, Mumbai

Collection and Authentication of Plant Material

Fresh roots of *Butea monosperma* were collected from Chhatrapati Sambhajinagar, Maharashtra. The plant material was authenticated by the Department of Botany, Padmavati Arts and Science College, Mohadi-Amdabad. The authenticated specimen was deposited with voucher number 1705.

Processing of Crude Drug

The collected roots were washed, shade dried and powdered. The powder was passed through mesh No.14 and stored in an airtight container for further studies.

Pharmacognostic Evaluation

The root was evaluated for morphological characteristics including colour, odour, taste, shape, surface and texture.

Physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, loss on drying and extractive values were determined according to standard procedures.

Extraction of Plant Material

Powdered roots were extracted by continuous hot extraction method using Soxhlet apparatus.

Three extracts were prepared:

- Petroleum ether extract
- Ethanolic extract
- Methanolic extract

The extracts were concentrated, dried and percentage yield was calculated.

Preliminary Phytochemical Screening

The extracts were screened for the presence of:

- Carbohydrates
- Proteins
- Amino acids
- Steroids
- Alkaloids
- Flavonoids
- Glycosides
- Tannins and phenolic compounds

Standard qualitative chemical tests were performed.

Quantitative Phytochemical Analysis

Total phenolic content was estimated by Folin–Ciocalteu method using gallic acid as standard.

Total flavonoid content was determined by aluminium chloride method using rutin as standard.

Antioxidant Activity

In-vitro antioxidant activity was evaluated by DPPH radical scavenging assay. Different concentrations of extracts were tested and absorbance was measured using UV-visible spectrophotometer.

Pharmacological Evaluation

Acute toxicity study was performed according to OECD guideline 423 and extracts were found safe up to 2000 mg/kg.

Anti-anxiety activity was evaluated using Elevated Plus Maze model.

Wistar rats (180–230 g) were used after IAEC approval.

Experimental Groups

Group I – Control (0.5% normal saline)

Group II – Diazepam 2 mg/kg

Group III – Ethanolic extract 100 mg/kg

Group IV – Ethanolic extract 200 mg/kg

Group V – Methanolic extract 100 mg/kg

Group VI – Methanolic extract 200 mg/kg

IV.RESULTS

Collection, Identification and Authentication of Plant Material

The root material of *Butea monosperma* was collected from Marathwada region, Maharashtra, India. The collected specimen was authenticated by a qualified botanist from Padmavati Arts and Science College, Mohadi-Amdabad, Tq. Kannad, Dist. Chh. Sambhajinagar, Maharashtra. The plant was identified as *Butea monosperma* belonging to family Fabaceae. Authentication certificate was issued and voucher specimen was deposited with accession number 1705.

Pharmacognostic Evaluation

The root of *Butea monosperma* showed brown to dark brown colour, characteristic odour, bitter and astringent taste, cylindrical irregular shape, rough surface and hard fibrous texture.

Table: Macroscopic Characteristics of *Butea monosperma* Root

Parameter	Observation
Colour	Brown to dark brown
Odour	Characteristic
Taste	Bitter and astringent
Shape	Cylindrical, irregular
Surface	Rough with longitudinal wrinkles
Texture	Hard and fibrous

Processing of Crude Drug

The collected root material was washed, shade dried, powdered and stored in an airtight container for further experimental studies.

Physicochemical Evaluation

The physicochemical evaluation confirmed the quality and purity of the plant material.

Table: Physicochemical Standardization Parameters

Parameter	Result
Ash value	4%
Acid insoluble ash	1.5%
Water soluble ash	8.5%
Loss on drying	5%

Extractive Value

Fig. 11: Extractive Values of *Butea monosperma* Root in Different Solvents

Table: Extractive Values of Different Solvents

Solvent	Extractive Value (% w/w)
Petroleum ether	7.5
Chloroform	8.5
Ethyl acetate	16.0
Acetone	22.5
Methanol	27.0
Ethanol	25.0
Water	10.5

Methanol showed the highest extractive value followed by ethanol, indicating better extraction of polar phytoconstituents.

Extraction of Plant Material

The powdered root was extracted by Soxhlet apparatus. Defatting was performed using petroleum ether followed by extraction with ethyl acetate and ethanol.

Fig. 12: Soxhlet Assembly

Preliminary Phytochemical Screening

The extracts showed the presence of alkaloids, flavonoids, glycosides, steroids, proteins, carbohydrates, tannins and phenolic compounds. Methanol and ethanol extracts showed higher phytochemical content.

Quantitative Analysis

Total Phenolic Content

Extract	Phenolic Content (mg GAE/g extract)
Methanol	28.40 ± 0.65
Ethanol	33.10 ± 0.72

Ethanol extract showed higher phenolic content.

Total Flavonoid Content

Extract	Flavonoid Content (mg RE/g extract)
Methanol	26.80 ± 0.60
Ethanol	30.40 ± 0.68

Ethanol extract showed higher flavonoid content.

Antioxidant Activity (DPPH Method)

The extracts showed concentration-dependent antioxidant activity. Ethanol extract showed higher DPPH scavenging activity compared to ethyl acetate extract due to higher phenolic and flavonoid content.

Pharmacological Screening

IAEC approval was obtained and Wistar rats were used for evaluation of antianxiety activity.

Grouping of Animals

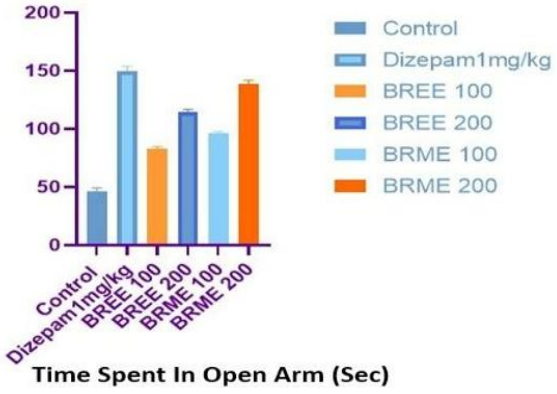
Group	Treatment	Dose
Control	Normal saline	—
Standard	Diazepam	2 mg/kg
BREE	Ethanolic extract	100 & 200 mg/kg
BRME	Methanolic extract	100 & 200 mg/kg

Elevated Plus Maze Test

The extracts showed significant anxiolytic activity. Methanolic extract (200 mg/kg) showed maximum activity with increased open arm entries and time spent in open arm, comparable with standard drug diazepam.

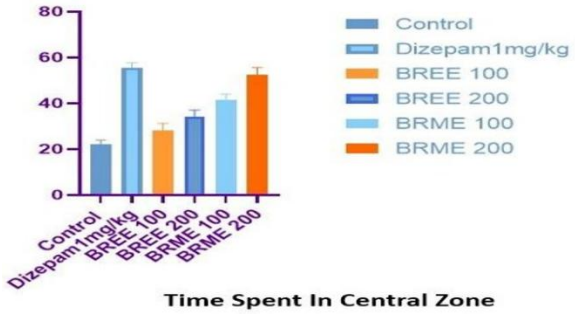
Graph 1

Graph 2



Graph 3

Graph 4



Graph 5

V.DISCUSSION

The present study was carried out to evaluate the phytochemical and pharmacological potential of *Butea monosperma* root extract for antianxiety activity in rats. The collected plant material was authenticated and evaluated for pharmacogenetic and physicochemical parameters, confirming the quality and purity of the crude drug.The extractive value showed higher yield in polar solvents, especially methanol (27%) and ethanol (25%), indicating the presence of polar bioactive constituents. Phytochemical screening confirmed the presence of alkaloids, flavonoids, glycosides, steroids, tannins and phenolic compounds, which may contribute to the therapeutic activity.

Quantitative analysis revealed higher phenolic and flavonoid content in ethanol extract, suggesting better antioxidant potential. DPPH assay confirmed significant free radical scavenging activity, with ethanol extract showing strong antioxidant activity .Acute toxicity studies showed that the extracts were safe up to 2000 mg/kg. The Elevated Plus Maze model demonstrated significant anxiolytic activity of both ethanolic and methanolic extracts. BRME 200 mg/kg showed maximum activity, comparable with diazepam.

The observed activity may be due to flavonoids, phenolics and alkaloids that possess neuroprotective and GABA-modulating properties. Overall, *Butea monosperma* root extract showed promising antioxidant and antianxiety potential.

Further studies are required for isolation of active compounds and clinical evaluation.

VI.CONCLUSION

The present study entitled “Phytochemical Investigation and Pharmacological Evaluation of *Butea monosperma* Root Extract for Antianxiety Activity in Rats” was carried out to evaluate the phytochemical composition, antioxidant potential, and anxiolytic activity of *Butea monosperma* root extracts. The study was designed to provide scientific evidence for the traditional use of this medicinal plant in the management of anxiety-related disorders.

The plant material was collected from the Marathwada region of Maharashtra and authenticated by a qualified botanist. Pharmacognostic evaluation revealed characteristic features of the root including brown to dark brown colour, rough surface, fibrous texture, bitter and astringent taste, which helped in identification and standardization of the crude drug. Physicochemical evaluation showed acceptable quality parameters with total ash value (4%), acid insoluble ash (1.5%), water soluble ash (8.5%), and loss on drying (5%), confirming the purity and suitability of the plant material for further investigations.

The extraction study demonstrated that polar solvents were more effective in extracting bioactive constituents. Methanol and ethanol extracts showed higher extractive values compared to non-polar solvents, indicating the presence of higher amounts of polar phytoconstituents. Preliminary phytochemical screening confirmed the presence of various secondary metabolites including alkaloids, flavonoids, glycosides, steroids, tannins, phenolic compounds, carbohydrates and proteins. These phytoconstituents

are known to possess important biological activities, including antioxidant and neuroprotective effects.

Quantitative estimation of phytoconstituents revealed that ethanol extract contained higher total phenolic content (33.10 ± 0.72 mg GAE/g extract) and flavonoid content (30.40 ± 0.68 mg RE/g extract) compared to methanol extract. The antioxidant activity evaluated by DPPH radical scavenging assay showed concentration-dependent activity. The ethanol extract exhibited significant antioxidant activity, which may be attributed to the presence of phenolic and flavonoid compounds capable of scavenging free radicals and reducing oxidative stress.

The safety evaluation of the extracts through acute toxicity studies showed no toxic effects or mortality up to 2000 mg/kg dose. Based on the safety profile, doses of 100 mg/kg and 200 mg/kg were selected for pharmacological evaluation. The antianxiety activity was assessed using the Elevated Plus Maze model, which is a well-established method for evaluating anxiety behaviour in experimental animals.

The results of the Elevated Plus Maze study demonstrated that both ethanolic and methanolic extracts of *Butea monosperma* root produced significant anxiolytic effects. Treatment with extracts increased the number of open arm entries and time spent in open arms, while decreasing time spent in closed arms compared to the control group. These changes indicated reduced anxiety-like behaviour in treated animals. Among all treatment groups, BRME 200 mg/kg showed the most significant activity, which was comparable with the standard drug diazepam.

The anxiolytic effect of *Butea monosperma* root extract may be associated with the presence of flavonoids, phenolic compounds and alkaloids. These phytoconstituents may influence neurotransmitter pathways, particularly GABAergic mechanisms, and provide neuroprotective effects by reducing oxidative stress. The combined antioxidant and phytochemical properties may contribute to the observed antianxiety activity.

Overall, the present investigation provides scientific support for the traditional medicinal use of *Butea monosperma*. The root extracts demonstrated significant phytochemical richness, antioxidant activity, safety and promising anxiolytic potential. The findings suggest that *Butea monosperma* root may serve as a valuable natural source for the development of herbal antianxiety agents.

However, further studies are required for isolation and identification of active constituents, detailed mechanism of action, long-term toxicity studies and clinical evaluation to establish its therapeutic application in humans. The present study concludes that *Butea monosperma* root possesses significant potential as a safe and effective herbal candidate for future development in anxiety management.

REFERENCE

[1] Thibaut F. The role of sex and gender in neuropsychiatric disorders. *Dialogues Clin Neurosci.* 2016;18(4):351-352.

[2] Thibaut F. Gender does matter in clinical research. *Eur Arch Psychiatry Clin Neurosci.* 2017;267(4):283-284.

[3] Wittchen HU, Kessler RC, Beesdo K, Krause P, Hofler M, Hoyer J. Generalized anxiety and depression in primary care: prevalence, recognition, and management. *J Clin Psychiatry.* 2002;63(Suppl 8):24-34.

[4] Bandelow B, Baldwin D, Abelli M, et al. Biomarkers for anxiety disorders, OCD and PTSD: a consensus statement part II. Neurochemistry, neurophysiology and neurocognition. *World J Biol Psychiatry.* 2017;18(3):162-214.

[5] Parsaik AK, Mascarenhas SS, Khosh-Chashm D, et al. Mortality associated with anxiolytic and hypnotic drugs: a systematic review and meta-analysis. *Aust N Z J Psychiatry.* 2016;50(6):520-533.

[6] Zhong G, Wang Y, Zhang Y, Zhao Y. Association between benzodiazepine use and dementia: a meta-analysis. *PLoS One.* 2015;10(5):e0127836.

[7] Dodds TJ. Prescribed benzodiazepines and suicide risk: a review of the literature. *Prim Care Companion CNS Disord.* 2017;19(2):doi:10.4088/PCC.16r02037.

[8] Vos T, Abajobir AA, Abbafati C, et al. Global, regional and national incidence, prevalence and

years lived with disability for diseases and injuries, 1990–2016: Global Burden of Disease Study. *Lancet.* 2017;390:1211-1259.

[9] Gustavsson A, Svensson M, Jacobi F, et al. Cost of disorders of the brain in Europe 2010. *Eur Neuropsychopharmacol.* 2011;21:718-779.

[10] Alonso J, Liu Z, Evans-Lacko S, et al. Treatment gap for anxiety disorders is global: results from World Mental Health Surveys. *Depress Anxiety.* 2018;35:195-208.

[11] Batelaan NM, Rhebergen D, de Graaf R, et al. Panic attacks as a dimension of psychopathology. *J Clin Psychiatry.* 2012;73:1195-1202.

[12] Lamers F, van Oppen P, Comijs HC, et al. Comorbidity patterns of anxiety and depressive disorders: NESDA study. *J Clin Psychiatry.* 2011;72:341-348.

[13] Scholten WD, Batelaan NM, Penninx BWJH, et al. Diagnostic instability and recurrence in depressive and anxiety disorders. *J Affect Disord.* 2016;195:185-190.

[14] Hendriks SM, Spijker J, Licht CMM, et al. Two-year course of anxiety disorders. *Acta Psychiatr Scand.* 2013;128:212-221.

[15] Hendriks SM, Spijker J, Licht CMM, et al. Long-term disability in anxiety disorders. *BMC Psychiatry.* 2016;16:248.

[16] Robins LN, Wing J, Wittchen HU, et al. The Composite International Diagnostic Interview. *Arch Gen Psychiatry.* 1988;45:1069.

[17] Spitzer RL, Williams JBW, Gibbon M, First MB. Structured Clinical Interview for DSM-III-R. *Arch Gen Psychiatry.* 1992;49:624.

[18] Ambrosini PJ. Historical development and present status of K-SADS. *J Am Acad Child Adolesc Psychiatry.* 2000;39:49-58.

[19] Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. *Acta Psychiatr Scand.* 1983;67:361-370.

[20] Meier SM, Petersen L, Mattheisen M, et al. Secondary depression in severe anxiety disorders. *Lancet Psychiatry.* 2015;2:515-523.

[21] Penninx BWJH, Nolen WA, Lamers F, et al. Two-year course of depressive and anxiety disorders. *J Affect Disord.* 2011;133:76-85.

[22] Pitman A, Suleman S, Hyde N, Hodgkiss A. Depression and anxiety in patients with cancer. *BMJ.* 2018;361:k1415.

[23] Eisner MD, Blanc PD, Yelin EH, et al. Influence of anxiety on health outcomes in COPD. *Thorax.* 2010;65:229-234.

[24] Copeland WE, Angold A, Shanahan L, Costello EJ. Longitudinal patterns of anxiety from childhood to adulthood. *J Am Acad Child Adolesc Psychiatry.* 2014;53:21-33.

[25] Ormel J, Raven D, van Oort F, et al. Mental health in Dutch adolescents: TRAILS study. *Psychol Med.* 2015;45:345-360.

[26] Kessler RC, Demler O, Frank RG, et al. Prevalence and treatment of mental disorders. *N Engl J Med.* 2005;352:2515-2523.

[27] Silove DM, Tay AK, Tol WA, et al. Separation anxiety symptoms and traumatic stress. *J Affect Disord.* 2016;205:292-300.

[28] Meier SM, Deckert J. Genetics of anxiety disorders. *Curr Psychiatry Rep.* 2019;21:16.

[29] Meier SM, Trontti K, Purves KL, et al. Genetic variants associated with anxiety disorders. *JAMA Psychiatry.* 2019;76:924.

[30] Purves KL, Coleman JRI, Meier SM, et al. Common genetic variation in anxiety disorders. *Mol Psychiatry.* 2019.

[31] Forstner AJ, Awasthi S, Wolf C, et al. Genome-wide association study of panic disorder. *Mol Psychiatry.* 2019.

[32] Otowa T, Hek K, Lee M, et al. Meta-analysis of genome-wide association studies of anxiety disorders. *Mol Psychiatry.* 2016;21:1391-1399.

[33] Levey DF, Gelernter J, Polimanti R, et al. Genetic risk loci for anxiety disorders. *Am J Psychiatry.* 2020;177:223-232.