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Animal models for antidepressant activity assay on natural and conventional agents: A review of preclinical testing

Ani Kristiyani[1,2], Zullies Ikawati[1*], Andayana Puspitasari Gani[3], Zaenal Muttaqien Sofro[4]

[1]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Indonesia
[2]Faculty of Pharmacy, Universitas Kristen Immanuel, Yogyakarta 55571, Indonesia
[3]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Indonesia
[4]Department of Physiology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281

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ABSTRACT

Depression is characterized by symptoms such as insomnia, change in appetite and body weight, impaired concentration, lethargy, agitation, psychomotor retardation, anhedonia, feelings of worthlessness, and suicidal thoughts. Various methods have been applied to assess the efficacy of antidepressants and associated molecular processes. However, guidance on the selection of methods for antidepressant testing is still lacking. This article provides a comprehensive review focusing on animal models of depression and the behavioral and molecular changes. A literature search was conducted on platforms, such as PubMed, Google Scholar, Scopus, and Science Direct to find relevant articles. This review found that chronic unpredictable mild stress might be the best and most commonly used model for inducing depression and simulating human depressive states in animals. This model employs a naturalistic approach to expose animals to unpredictable stressors that disrupt homeostasis and cause somatic, physiological, neurobiological, and biochemical disorders and behaviors. The forced swim test (FST) is the most frequently used simple behavioral testing method that is consistent with antidepressant effects, reduces immobility time, and serves as the leading indicator of antidepressant effectiveness. The most commonly observed molecular changes are those related to the levels of monoamine neurotransmitters, such as 5-hydroxytryptamine, dopamine, norepinephrine, and brain-derived neurotrophic factor (BDNF), which are the main etiological contributors to depression. The findings from this review will contribute to ongoing efforts to discover and develop drug candidates for the treatment of depression.

Implication for health policy/practice/research/medical education:
This review highlights the preclinical methods used in research on antidepressants in animals to observe behavioral and molecular changes. The results will serve as a valuable method development guide for researchers.
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Introduction

Depressive disorders affect mental and physical pain, causing significant emotional distress. Symptoms of depression include depressed mood, marked reduction or even loss of interest or pleasure, substantial weight gain or weight loss despite not dieting, psychomotor agitation or retardation, fatigue or loss of energy, excessive or inappropriate worthlessness or guilt, diminished ability to think or concentrate, and recurring thoughts of death (1). Depression is influenced by genetic, biological, and environmental factors. Neurotransmitters such as dopamine (DA), 5-hydroxytryptamine (5-HT), and norepinephrine (NE), as well as amino-gamma-butyric acid, glutamate (Glu), various neurochemicals, hormones, pro-inflammatory cytokines, and other unidentified cytokines play interrelated roles in the process of depression (2). Depression has a long history throughout the world, and significant recent research has been dedicated to the

***Corresponding author**: Zullies Ikawati,
Email: zullies_ikawati@ugm.ac.id

development of antidepressants (3). The development of a valid animal model of depression is challenging due to the unique and complex nature of depression in humans. Current models of depression are critically evaluated for ease, validity, and replicability using various behavioral tests. Most animal models of depression aim to test physiological behaviors quantitatively (4). Behavioral tests in animals have been developed to verify the theories of cognition, emotion, and psychopathology. Several tests have been used for preclinical research on depressive behavior (5). Meanwhile, molecular changes that occur in animals after exposure to stress or antidepressants can be further explored and validated to increase knowledge of biomarkers in depression (4). Guidance on the selection of methods for natural and conventional antidepressant testing is still lacking. In this regard, the review articles on herbal activities for depression have only covered aspects such as the names of the herbs, active parts, types of extracts used, major active constituents, dosage, pharmacological activity, and molecular mechanisms (6–8). There is a need for a review of methods to test natural or synthetic compounds as antidepressants. This article aims to summarize and focus on preclinical research on antidepressant activity in animal models of depression, with behavioral and molecular changes as variables, to serve as a reference for future research.

Material and Methods

This review was conducted on relevant original articles from reputable databases such as Google Scholar, Scopus, and Science Direct, with "herbs or plants", "depression" and "preclinical or rodent studies or mice or rats" as the keywords. The search was limited to English full-text articles published within the last two years, from 2022 to October 2023, comprising original research articles on antidepressants and other relevant articles. This review excluded review articles, conference articles, theses, dissertations, and articles whose data were unavailable for retrieval. Variables evaluated in this review are stress models, behavioral tests, and molecular changes.

Results

The literature search retrieved 742 articles from the databases, of which 71 were identified as relevant for inclusion. This review focuses on the evaluation of models employed in research to test antidepressant activity, with parameters including stress models, behavioral tests, and molecular changes. Table 1 summarizes of stress model interventions applied to animals in research on antidepressant activity.

Widely used stress-induced animal models of depression

Chronic unpredictable mild stress (CUMS)

The CUMS model has been modified by subjecting animals to various stressors, such as heating at 45 °C for 5 minutes, exposure to predators for 15 minutes, fasting for 24 hours, restraint for 30 minutes, placement on wet bedding for 8 hours, forced swimming at 4 °C for 5 minutes, cage-shaking for 8 hours, exposure to white light strobe stimulation for 8 hours, exposure to noise stimulation for 8 hours, placement in an empty cage with water 1 cm from the bottom, alteration of the light/dark cycle, placement in a cage containing wet sawdust, and cage-tilting at a 30-degree angle. In this model, mice are randomly exposed to various stressors every day for 8 weeks to induce a state of social stress (9,10,12,36,38). CUMS causes somatic, physiological, neurological, biochemical, and behavioral abnormalities by disrupting homeostasis. Susceptibility to CUMS-induced anhedonia is associated with reduced gene expression and changes in dopaminergic neurons of the ventral tegmental area (VTA) and the hypothalamic-pituitary-adrenal (HPA) axis (4). The CUMS model is considered the most superior and widely used animal model of depression to replicate human conditions. However, this model is susceptible to minor changes in design (10,12). To reduce this susceptibility, improvements to the CUMS protocol are needed to take into account crucial factors such as chronic sleep deprivation, painful stressors, social stressors, differences in sex and age of the animals, variations in stress tolerance among animal strains, quality of handling, habituation to stressors, and various combinations of physical and psychological stress (70).

Forced swim test (FST)

In FST, mice are evaluated based on their swimming behavior. Mice are placed in transparent cylinders of approximately the same size (17 cm in diameter and 20 cm in height) containing water at a temperature of ±23 °C and a water level of 13 cm, in dim lighting conditions. Parameters of the floating behavior, including the number of immobility episodes, are recorded (38). FST can decrease serotonergic neurotransmission, resulting in prolonged immobility. This may lead to psychomotor impairments and long-term adaptive changes in neural circuits that underlie antidepressant effects. Stress-induced immobility is regulated by a diversity of genes, including transcription factors, endocrine hormones, and immunity (4). FST is relatively affordable and easy to perform, and the results can be analyzed quickly and easily. However, this method also has several disadvantages, such as the problem of chronic augmentation and the potential impact on brain structure/function if brain analysis is performed afterward (70).

Tail suspension test (TST)

In TST, the tail of the mouse subject is raised above the surface, thus preventing its ability to escape or grab onto nearby surfaces. The implementation of this method only requires a suspension device or shelving unit along with adhesive material. Similar to FST, TST also decreases serotonergic neurotransmission, resulting in immobility.

Table 1. Summary of stress model interventions applied to animals in research on antidepressant activity

Stress model	Duration of stress	Observed stress	Reference
CUMS	21-56 days	Anhedonia, immobility, decrease in the sucrose preference, changes in locomotor activity, changes in body weight	(9-36)
FST	6-30 min	Immobility	(37-49)
TST	6 min	Immobility	(37,40,41,43,44,47,49-53)
LPS	on days 1, 4, 9, and 14	LPS-induced depression-like behaviors, production of ROS in HT-22 cells, proinflammatory cytokine release.	(25,54,55)
MS	7 days	Changes in behavior caused by the separation of the child from the mother	(56,57)
SHS	3 days	Social defeat	(58)
Emotional US	21 days	US-induced behavioral changes and blood cortisol increases	(38)
Induced by ovariectomy	-	Ovariectomy-induced behavioral changes	(59)
CSDS	10 days	Defeat against intruder	(60,61)
pCPA (300 mg/kg, i.p.)	Once a day for seven days	Locomotor activity	(62)
Isoproterenol (85 mg/kg, s.c.)	1 time a day for two days	Isoproterenol induced behavior changes, functional, biochemical, histopathological alterations in the heart tissue, necrosis of myocardial muscles	(63)
Intraperitoneally injected with CORT 20 mg/kg	1 time	Changes in behavior	(64)
CRS	21 days	Behavior changes, lipid absorption deficit, reduced body weight, circulating lipid level	(57,65)
AlCl3 (300 mg/kg, i.p.)	1 time	Changes in behavior	(66)
SD stress	72 h	Changes in behavior	(67)
Reserpine (0.5 mg/kg, i.p.)	1 time	Changes in behavior	(14)
Olfactory bulbectomy	-	Changes in behavior	(68)
ARS	240 min	Changes in behavior, locomotor activity, social interaction	(69)

CUMS: Chronic unpredictable mild stress; FST: Forced swim test; TST: Tail suspension test; LPS: Lipopolysaccharide; MS: Maternal separation; SHS: Social hierarchy stress model; US: ultrasound stress; CSDS: Chronic social defeat stress; pCPA: Para-chlorophenyl-alanine; CRS: Chronic restraint stress; AlCl3: Aluminum chloride; SD: Sleep deprivation; ARS: Acute restraint stress; ROS: Reactive oxygen stress; CORT: corticosterone.

Utilization of this method offers several benefits, including its compact size, cost-effectiveness, ease of use, and the possibility of automating data collection through electromechanical measurement systems and video analysis. However, TST is not recommended to be applied to larger rodent species (e.g., rats) as it may cause discomfort due to the entire weight of the body resting on the tail. In addition, TST is not suitable for certain strains of mice, particularly the C57BL/6 strain, which tend to escape from captivity by climbing their own tails. Therefore, these mice must be excluded from the analysis process, thus increasing the number of mice needed for the experimentation (71).

Lipopolysaccharide (LPS)

In this test, a depression-like model is induced by intraperitoneal injection of LPS (600 μg/kg, i.p.). The mice are acclimatized in the experimental room for 2 hours, after which they are given an antidepressant or a control substance. One hour later, another dose of LPS (600 μg/kg, i.p.) is administered to the mice, and behavioral tests are performed at 6 and 24 hours after LPS administration (55). At the molecular level, LPS is a potent inducer of tumor necrosis factor alpha (TNFα), interleukin (IL)-1β, and 5-HT2A messenger ribonucleic acid (mRNA) expression in specific brain regions. A limitation of LPS is that this method may not cause noticeable behavioral changes in depression tests (72).

In existing research, the CUMS model is the most commonly used stress induction method. However, it is also considered the most challenging method as it is time-consuming and requires a lot of equipment. CUMS applies a naturalistic approach to expose animals to unpredictable stressors that disrupt homeostasis and cause somatic, physiological, neurobiological, biochemical, and behavioral disturbances. Anhedonia is one of the

major consequences of CUMS (70). This stress model is susceptible to changes in experimental design; the better the procedure is standardized the more factors can be controlled. Despite its complexity, CUMS seems to be one of the best models for simulating human depressive states in animals (70).

Behavioral tests

Behavioral tests in experimental animals are done to assess the extent to which the rat model exhibits depression-like symptoms. Various methods have been developed to measure depressive behavior in experimental animals, each of which presents different results. Behavioral testing is used in research as a key investigative tool to reveal the activity of a drug against depression. Table 2 provides a brief explanation of the methods used in these behavioral tests.

Forced swim test

A 20 × 35 cm (dxh) glass cylinder is used in FST, with a water level in the cylinder of approximately 20 cm and a water temperature of ±23 °C. The movements of the mice are recorded for 6 minutes. The duration of immobility, referring to instances of floating without any movement, is measured over 4 minutes, from the second to the sixth minute. The time taken for the subjects to start floating is also measured (in minutes). Immobility is defined as the mice not moving and keeping their head above water. It is identified as a state of "desperation"; and the reduction of immobility is used as a parameter of antidepressant activity. FST is the most widely employed test to evaluate the effects of antidepressants because it is fast, cheap, and easy to analyze. However, this method has weak validity values and numerous confounding variables, such as tool size, temperature, and water depth (4,37–39,50,51,58).

Open field test (OFT)

OFT is used to determine behavioral responses to stress, especially those caused by central and peripheral infections as well as changes in cytokine levels (IL-6, IL-10) that produce adaptive responses. This method assesses the locomotor activity of mice by observing their movements in a 100 × 100 × 100 cm box illuminated with light either from the ceiling or near the walls. The mice are positioned in the center of the box, and the frequency with which they enter the box as well as the number of defecations are documented. Before the experiment, the open field area is sterilized with a 70% ethanol solution (38,58). Observable metrics in this test include crossing, rearing, grooming, and defecation (55). OFT is considered a standard, rapid, and inexpensive test. However, the behavior of the mice is influenced by several parameters, such as their gender

Table 2. Behavioral test methods used on animals in antidepressant research

Behavioral tests	Observed behavior	Reference
FST	Immobility, index of immobility, swimming time, climbing time	(10,12,14-17,19-27,30,32-43,46-49,54,56-68,73-80)
OFT	Locomotor activity (total distance moved, time in zone periphery, time in zone center)	(9–12,15,17–21,23,25,28,29,31,34-36,38,42,44,48,49,55–59,61–65,68,73,81)
TST	Immobility	(9,11,12,16,17,19,22,25,26,32,36,37,39-41,43,44,49,51-53,55,57,58,60,62,64,67,74,76,77,79,82)
SPT	Sucrose consumption	(11,13–16,18–23,25,28,29,32,35,36,54,56-58,61,64,77,82)
EPM	Open arm time	(9,23,33,35,43,54,57,58)
NSF	Latency, feeding time	(16,30,36,83)
LDT	Latency, time in light box, locomotor activity	(39,48,69)
FUST	Sniffing time	(29,58)
EOM	Latency to float, number of exits to open arms	(38)
ILT	Latency	(13)
NBT	Nest score in the nest building test	(65)
YMT	Cognitive deficits, total number of entries	(76)
PAT	Latency time	(77)
HBT	Number of head dips	(45)
WCFC	Body weight, food intake	(57)
Body weight	Body weight	(82)
CPE	Pole climbing score	(31)
SIT	Social interaction ratio	(61)

FST: Forced swim test; OFT: Open field test; TST: Tail suspension test; SPT: Sucrose splash test; EPM: Elevated plus maze; NSF: Novelty-suppressed feeding; LDT: Light-dark test; FUST: Female urine sniffing test; EOM: Elevated o-maze; ILT: Ingestion latency test; NBT: Nest building test; YMT: Y maze test; PAT: Passive avoidance test; HBT: Hole board test; WCFC: Weight change and food consumption; CPE: Climbing pole experiment; SIT: Social interaction test.

and the size and shape of the instrument used. In addition, OFT is not used specifically for depression testing but rather for anxiety testing (5,84).

Tail suspension test

In TST, each mouse is suspended 45 cm above the floor with adhesive tape placed approximately 1 cm from the tip of its tail. The movements of the mice are videotaped for 6 minutes, and their immobility is measured by reviewing the video recordings. Immobility is defined as the absence of limb movement (51,55,58). Instances where the mouse climbs on its tail are excluded. Total immobility is recorded during the last 4 minutes of the 6-minute duration (37). TST is one of the most widely used tests to evaluate the effects of antidepressants because it is fast, cheap, and easy to analyze. Nonetheless, this method can only be applied to mice whose body weight is smaller than that of rats. In TST, motor deficits affect the test results (84).

Sucrose preference test (SPT)

SPT calculates the anhedonia experienced by mice with depression. Anhedonia and hopeless behavior are used to test the efficacy of antidepressants based on the construct validity of depressive symptoms which affects the inhibition of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA receptor) antagonists and the phosphorylation of mammalian target of rapamycin (mTOR) and glutamate receptor 1 (GluR1) in the prefrontal cortex and hippocampus. In this test, mice are acclimated by being given two bottles containing a standard sucrose solution (1%) for 24 hours. Over the next 24 hours, one bottle of sucrose solution is replaced with regular water. Subsequently, the mice are left for another 24 hours without access to food or water. In another procedure of SPT, the mice are exposed to two bottles, each containing sucrose solution and plain water, continuously for 12 hours. Volumetric measurements of each bottle are documented both before and after the experiment. SPT is carried out at night (58). SPT testing in healthy mice did not increase sucrose preference (85). SPT is one of the most common tests to assess anhedonia which is cheap, quick, and simple. The assessment and measurement methods of each laboratory may differ, and the state of anhedonia in humans is not always associated with changes in the consumption of sweet solutions, meaning that SPT cannot extrapolate results observed in animals to humans (84,86).

Elevated plus maze (EPM)

The experimental setup for EPM consists of an opaque white cross-shaped Plexiglas maze positioned at a height of 40 cm. Each arm measures approximately 30 × 5 × 30 cm. Two arms in opposite positions contain openings and the other two arms contain closures. The mouse is placed in the center of the maze with its head facing the open arm. Mice movements are recorded for 5 minutes, and the time spent in the open arm is calculated. Depressed mice tend to avoid open spaces; this depressive behavior is related to the 5-HT1A receptor which plays a role in regulating mood (58). EPM is easy to use, and results can be obtained within 5 minutes per experimental animal. However, this method is more often used to test anxiety than depression. EPM is a behavioral test widely used in rodents and has been validated to assess anti-anxiety effects (84,87).

Novelty-suppressed feeding test (NSFT)

NSFT is a test that involves the inhibition or control of feeding behavior. In this test, mice undergo a 24-hour fasting period prior to the experiment. Food pellets are placed in a specific location, measuring 60 × 60 cm, in a Plexiglas container under low lighting conditions (<20 lux). Latency is assessed within a 5-minute timeframe, where latency is calculated as 5 minutes if the mice do not eat within this period. Behaviors such as touching and smelling the food are not classified as eating. Reduced latency is a form of stress and can lead to a decrease in phospho-mTOR in the prefrontal cortex, a molecular signal associated with acute depression (16,37). This test lacks a strict consensus on its methodology, thus reducing its validity and reproducibility as well as complicating its translation. Nevertheless, this assay has recently been used to study chronic and sub-chronic antidepressant treatment in rodent models (5,88).

Female urine sniffing test (FUST)

In FUST, mice are acclimated to aseptic cotton-tipped applicators inserted into their cages for 1 hour. Each mouse is then exposed to two cotton-tipped applicators, one soaked in water and the other soaked in the urine of estrous females, in a quiet and dimly lit environment (<20 lux). The experiment lasts 3 minutes, during which the amount of time the mice spend sniffing each cotton tip is measured, with depressed mice having less sniffing time (58). In male mice, sniffing the urine of estrous females is a preferred activity, and stress reduces this behavior. When sniffing the urine of estrous females, NAc dopamine levels increase significantly. This test is highly predictive for examining stress resilience and vulnerability as well as the neurobiological substrates. However, FUST is sensitive to behavioral and genetic manipulation (89).

Elevated o-maze

The Elevated o-maze apparatus has a circular path with a base width of 5.5 cm and a diameter of 46 cm, positioned 45 cm above the floor. The two opposing arms are shielded by a wall, forming an enclosed area with a height of 10 cm. Illumination in this area is measured to be 25 lux. To ensure precise control of lighting conditions during the test, the apparatus is placed on a dark surface. Mice are placed in one of the enclosed arms of the apparatus. The delay between the first exit from the door and the entrance to the open compartment of the maze, as well as the number

of exits made into the open arm, are evaluated. Depression is assessed based on the latency time of the first entrance into and exit from the open compartment. Concentrations of IL-1β, mRNA, and malondialdehyde (MDA) and expression of IL-15, Akt, and glycogen synthase kinase-3 (GSK-3α) genes are significantly correlated with the assessment of duration in the maze (38). The advantage of applying elevated o-maze is the fast testing time (the results are obtained within 5 minutes), ease of assessment, and no disease risk in experimental animals. Apart from being a behavioral test, elevated o-maze can also be used to study brain locations and mechanisms (e.g., serotonin, GABA, Glu, etc.). The weakness of this test is that it is validated as an anti-anxiety test, not a depression test, and if it is applied more than once, the activity of the experimental animal will decrease on the second exposure (87).

Light-dark test (LDT)

LDT assesses animal's avoidance of brightly lit areas and its natural tendency to explore a new habitat. In this test, the mouse is first placed in a darkly lit enclosure. Then, the door separating the two compartments is opened. The latency to reach the lightbox and the time spent in each compartment are measured automatically. The test lasts approximately 8 minutes and is monitored using a video system. The time spent in the light compartment is considered anxiolytic activity. This correlates with plasma levels of corticosterone (CORT), dopaminergic system, and serotoninergic system that play an important role in the physiopathology of mood disorders (39). This tool is fast and easy to use, without requiring prior animal training. The weakness of this test is that it is influenced by tension, body weight, and age of the mice. LTD is more often used to test anxiolytics or drugs that act on serotonergic or neuropeptide receptors (90).

Ingestion latency test

In the ingestion latency test, the experiment is conducted for two days. The first day serves as an adaptation period, during which the mice are placed in an open square box for 10 minutes to acclimate. The test is carried out after a 24-hour fasting period. Food pellets are placed in the center of the exposed container and the mice are put back to assess the pellets. Each time the experiment is conducted the mice are placed in a consistent position and orientation. The time taken from the moment the mice are placed in the cage to the first food consumption is documented. Depressed mice have decreased activity and disruption of specific intestinal microorganisms (13). This method is rarely used to test antidepressant activity. However, it can be used to investigate antidepressant mechanisms based on intestinal flora, signifying that intestinal flora influences not only the gastrointestinal tract but also the function and behavior of the central nervous system through the gut-brain microbiota axis (13).

Nest building test (NBT)

In NBT, ten 7×5 cm pressed cotton 'nestlets' weighing a total of 24 g are placed in the center of the cage floor. The degree to which the rat bites the nestlets, moves them to the corner, and uses them to make its nest overnight is scored from 0 to 5. A score of less than 2 is considered indicative of a disease associated with neuroinflammation and depression with mechanisms involving regulation of nuclear factor-kappa B (NF-κB) translocation and the CX3C motif chemokine receptor 1 (CX3CR1/Nrf) pathways (65).

Hole-board test (HBT)

In HBT, the animal is placed in the center of a hole-board apparatus and given unrestricted access to explore the area for 5 minutes. The quantification of head dips is documented in a video. A head dip occurs when both eyes are fixated on the hole. Pocking the nose into a hole is a typical behavior in mice indicating curiosity, sensitivity to changes, and a fearless emotional state that links central nervous system (CNS) activity to depressant effects (45). Despite being rarely used as a sole assessment tool for testing new antidepressants, HBT is a behavioral testing method developed as a complement to other assays (91).

Weight change and food consumption (WCFC)

In this test, the weight of the tested mice is measured biweekly. The mice's daily food intake is closely monitored over several consecutive days, with precise measurements of food consumption taken at specified intervals. Depressed mice have impaired fat absorption, thus decreasing body weight and lipid levels (57). WCFC is easy to use, fast, and does not require complicated equipment. This test is commonly used to assess the effects of CUMS which requires the same regimen according to the time or age of the mice. However, WCFC cannot detect differences in weight gain between control mice and CUMS-induced mice. In addition, the lack of CUMS effects may present confounding results. Depression in humans can be accompanied by weight gain or loss and weight disorders. These changes in body weight are caused by CUMS and depend on the duration, timing, and type of stressor used. Therefore, CUMS-induced depression testing for weight changes should not be the primary parameter in antidepressant testing (92).

Climbing pole experiment (CPE)

The equipment used in CPE consists of a wooden rod measuring 3 cm in diameter and 760 mm in height. Mice are instructed to descend naturally from the top of the wooden rod, with their heads facing down. The duration of the climb to the base of the wooden rod is documented and the behavioral scores are determined. Mice are tested three times, and the mean score is calculated. Depression is shown in mice that experience tremors, quadriplegia, immobility, or death associated with changes in the levels

of DA, 5-HT, Glu, and gamma-aminobutyric acid (GABA) in the cerebrospinal fluid (31). CPE can be performed to test pain-related antidepressants and the efficacy of mu-opioid receptor (MOR) ligands as determinants of the effectiveness of MOR agonists in blocking stressor-induced climbing depression. In CPE, the experimental animal is at risk of suffering pain (93).

Behavior tests have been developed to verify and support cognitive, emotional, and psychopathological theories. Behavioral tests such as anxiety and depression tests often employ similar methods, including the OFT, LDT, and NSF tests (73). The FST is the most frequently used behavioral test method for assessing depression. FST is a simple method in which the animal is forced to swim with no possibility of escape, resulting in complete immobility except for the minimal movements necessary to keep the head above water. This easily recognizable state of immobility is interpreted as desperation or hopelessness. The methods used to induce this state are consistent with the effects of antidepressants which reduce immobility time and serve as a primary indicator of their effectiveness (73).

Molecular changes

Previous research has demonstrated the complexity of the multiple pathways associated with depression. The observed molecular changes include changes in monoamine levels, BDNF, CORT, genetic factors, and inflammation. Table 3 summarizes various molecular changes for assessing antidepressant activity.

Levels of serotonin (5-HT), noradrenaline (NA), DA, 5-hydroxyindoleacetic acid (5-H1AA), and homovanillic acid (HVA)

Examination of monoamines can be carried out using high-performance liquid chromatography coupled with electrochemical detection (HPLC-EC) and enzyme-linked immunosorbent assay (ELISA) as per the guidelines provided by the manufacturer. Samples can be obtained from the frontal cortex (FCx), striatum (Str), hippocampus (HPC), hypothalamus, and limbic areas (51). While depression is caused by decreased monoamine function in the brain, research in rodent models of stress suggests that increased DA and NA transmission may have a maladaptive role in stress-related disorders. Several recent drugs are still designed to increase monoamine transmission through inhibition of degradation or neuronal reuptake. Monoamine-based agents are currently considered potent antidepressants but alterations in central monoamine function also contribute to genetic susceptibility (94).

Determination of brain-derived neurotrophic factor (BDNF)

BDNF levels are measured in mouse serum and specific brain regions, including limbic areas, cerebral cortex, and hypothalamus. This is done using an ELISA kit according to the manufacturer's instructions. Data are quantified in units of nanograms per microgram (ng/μg) of protein (50,51). Exposure to stress shows a decrease in BDNF levels in the hippocampal, cortical, and subcortical regions. BDNF has been studied as a biomarker of major depressive disorder. The role of BDNF in the neurobiology of major depression may promote neuronal survival, neurogenesis, neuronal differentiation, and neuroplasticity. Since it is also associated with the modulation of memory and mood, BDNF is often used in antidepressant tests as an observed variable (95).

CORT and adrenocorticotropic (ACTH) hormone

CORT levels can be measured using the CORT ELISA kit, with specimens being extracted from either blood serum or hippocampus. The measurement of ACTH and CORT levels in serum is performed using commercially available kits, following the instructions from the manufacturer (11,17). In experimental animals, elevated cortisol is more often associated with acute stress and major depression rather than mild or atypical depression. Although almost all current treatments for major depression affect cortisol levels, a convincing relationship has not been found between cortisol levels and therapeutic response in either clinical or preclinical settings. Therefore, absolute cortisol levels cannot reliably predict the efficacy of antidepressant treatment (96).

Expression of cyclic adenosine monophosphate (cAMP) response element binding protein (CREB)

The phosphorylated cAMP response element binding (pCREB)-ser133, BDNF, mTOR, Akt, LC3, Beclin-1, p62, ERα, Erβ, and β-actin are detected by Western blotting using standard operating procedures (59). Chronic antidepressant treatment increases the expression of cAMP response element binding protein (CREB) in the rat hippocampus, making CREB a potential molecular target for novel therapeutic agents (97).

Determination of proinflammatory cytokines

TNF-α and IL-6 levels can be calculated using ELISA kits by utilizing serum, cerebral cortex, and hypothalamus samples from mice, following the manufacturer's instructions. The levels of TNF-α and IL-6 are obtained by adjusting protein concentrations. The data are quantified in picograms per microgram (pg/μg) of the protein (38). Major depression is accompanied by immune dysregulation and activation of the inflammatory response system. Concentrations of the proinflammatory cytokines TNF-α and IL-6 are significantly higher in depressed subjects compared to the control. Proinflammatory cytokines may contribute directly to the development of depressive symptoms. They have been shown to induce changes in stress-reactive neuroendocrine and central neurotransmitters in depression (98).

Table 3. Proposed mechanisms and molecular changes that occur in animals in studies of antidepressant activity

Proposed mechanisms	Molecular changes	Reference
Blockade on mTOR, GluR1	↑ Phosphorylation of mTOR expression ↑ Dephosphorylation GluR1 S845	(6,58,59)
Microarray analysis of OXT, OXTR	↑ Central OXT, OXTR expressions, and OXT protein levels	(51)
Determination of BDNF	↑ BDNF level	(9,16,21,50,51,59,60,63,67,69,77)
Determination of monoamine neurotransmitter and metabolite level	↑ DA, NA, Adrenaline, 5-HT, 5-HT1A ↓ DOPAC, HVA, 5-HIAA	(9,17,19,20,39,50–52,62,63,73)
Determination of oxidative stress and antioxidant activity	↓ MDA concentration, TAC, acetylation of SOD2 ↑ SOD, CAT, GSH	(10,38,74)
Determination of Protein Carbonyl	↓ Protein carbonyl	(38)
The gene expression	↓ mRNA expression of DRD1, DRD2, SERT ↑ regulated expression of neurotransmitter-related gene ↓ regulated expression of neuroinflammatory-related gene	(39,50)
Determination of proinflammatory cytokines and inflammatory response	↓ Level TNF-α, IL-6 ↓ TLR 2 gene expression, TLR4 gene expression, NF-κB gene expression, NLRP3	(18,20,28,50,63,67,74)
Determination of pCREB/CREB, Akt, LC3-II/LC3-I, Beclin-1, and p62 levels	↑ Levels of pCREB/ CREB ↑ Level of Akt ↓ Level of light chain 3 (LC3-Ⅱ/LC3-Ⅰ) ↓ Level of Beclin-1 ↑ Level of binding protein (p62)	(16,59,60,67)
Determination of DCX/ β-actin	↑ DCX/β-actin protein ratio	(60)
Determination of GABA	↑ Level GABA	(59,60)
Determination of cAMP	↑ Level of cAMP	(59,60)
Morphology of synaptic structures, regulating mitochondrial function	↑ Hippocampal synaptic plasticity ↑ positive areas (%) of postsynaptic density (PSD-95) and SYN ↓ ATP and reverse the expression levels of mitochondrial fusion/ fission proteins (Mfn2, Drp1, and Fis1)	(56)
Determination of protein level of Sirt3	↑ Protein level of Sirt3	(10)
Anandamide quantification	↑ Anandamide	(11,42)
Determination of CORT, ACTH, CRH	↓ Levels of CORT, ACTH, and CRH	(11,17)

↑: increased; ↓: suppressed; PFC: prefrontal cortex; HC: hippocampus; BDNF: Brain Derived Neurotrophic Factor; LC: Limbic system; GluR1: glutamate receptor 1; mTOR: mammalian target of rapamycin; OXT: Central Oxytocin; OXTR: oxytocin receptor; Oxytocin; DA: Dopamine; NA: Noradrenaline; FCx: the frontal cortex; Str: striatum; TNF-α: Tumor Necrosis Factor Alpha; IL: interleukin; CC: cerebral cortex; DOPAC: Dihydroxyphenylacetic acid; HVA: Homovanillic acid; 5-HIAA: 5-hydroxyindoleacetic acid; mRNA: Messenger ribonucleic acid; DRD: dopamine receptors; SERT: Serotonin Transporter; MDA: Malondialdehyde; SOD: superoxide dismutase; CAT: Catalase; TAC: Total antioxidant capacity; pCREB: Phosphorylated response element binding; GABA: Gamma-aminobutyric acid; GSH: glutathione; CORT: Corticosterone; ACTH: adrenocorticotropic; CRH: Corticotropin-releasing hormone; ATP: Adenosine triphosphate; Sirt3: Sirtuin3; SYN: synaptophysin; cAMP: cyclic adenosine monophosphate; TLR: Toll-like receptor; NF-κB: nuclear factor-kappa B; NLRP3: NOD-like receptor protein 3; SOD2: superoxide dismutase 2.

Changes in gene expression

The expression of several genes can be assessed by quantitative reverse transcription PCR (RT-qPCR). Trizol is used to extract total RNA from FCx, Str, and HPC. Spectrophotometry is used to measure the purity and quantity of RNA for each sample. Genes encoding the 5-HT receptor and 5-HT transporter in the 5-HT pathway, DA receptor, catechol-O-methyltransferase enzyme, and DA transporter in the DA pathway, and the monoamine oxidase that degrades 5-HT and DA are analyzed. The data are examined using the comparative threshold cycle approach and the results are reported as relative fold changes using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a housekeeping gene (39). The combination of various risk factors, such as genetic, environmental, and psychological factors, as well as the immune system, stress response, brain neuroplasticity, and neurotransmitter regulation, is suspected to lead to the development of major depressive disorder. Specific biomarkers can predict treatment response and provide real-time information regarding the status of biological factors that may influence treatment response (99).

MDA concentration

MDA is a biomarker that signifies the presence of lipid peroxidation (LPO) resulting from lipid oxidation. A decrease in MDA level indicates suppression of LPO in the brain (48). As an indicator of LPO, the level of MDA in the hippocampus is present in significant amounts in brain tissue. The tissue is rinsed briefly with cold PBS and disrupted in a lysis solution. Then, the resulting mixture is spun at 13 000 g for 10 minutes. TBA reagent is added

to the supernatant and incubated at 95°C for 60 minutes. After that, the supernatant is examined at a wavelength of 532 nm using a 96-well microplate (38). Mood regulatory pathways are influenced by oxidative damage to lipids. MDA measurement in blood samples is relatively easy and inexpensive. MDA may be a good candidate biomarker for major depression. As the end product of LPO, MDA can be used as a marker of oxidative stress. Reactive oxygen species (ROS) are involved in numerous neuropsychiatric diseases because ROS can damage the brain due to its high metabolic rate. Since MDA is a biomarker for oxidative stress, increased MDA in serum will escalate the risk of depression (100)

Superoxide dismutase (SOD) and catalase (CAT) concentration

SOD activity in the brain is assessed by measuring the reduction of nitroblue tetrazolium (NBT), which results in the formation of a water-insoluble molecule called blue formazan. The measurement of SOD activity is expressed as units per gram of brain tissue. The enzyme concentration required to achieve a 50% reduction in nitroblue tetrazolium reduction is defined as 1 unit. The generation of peroxyl radicals occurs through activation of the SOD enzyme which converts free radicals into hydrogen peroxide (H2O2). CAT activity is assessed by combining 0.1 mL of brain homogenate with a reaction mixture consisting of 1 mL of 0.01 M phosphate buffer at pH 7.4 and 0.4 mL of 0.2 M H2O2. The measurement of CAT activity is documented as the rate of H2O2 consumption in moles per minute per gram of brain tissue. An increase in SOD activity signifies the production of H2O2, which is subsequently accelerated by CAT, thus establishing a positive correlation with an increase in CAT activity. SOD and CAT provide the primary defense system to fight oxidative stress. Behavioral changes are caused by cofactor-dependent enzyme activation that is enhanced by antioxidant enzyme activity (48).

Total antioxidant capacity (TAC)

TAC is measured using a TAC assay. The color shift of the chelating chemical serves as an indicator of the antioxidant capacity of the substrate. A plate reader is used to measure absorbance at a wavelength of 570 nm. The relative TAC of the samples is determined using a standard curve (10). When oxidative stress is excessive, the body's TAC decreases. Stressful conditions lead to excessive ROS formation which in turn causes oxidative stress. Free radicals initiate a cascade, resulting in lipid peroxidation, DNA damage, cell death, and neurological problems. Plasma TAC is measured as an indicator of oxidative stress (101).

Anandamide quantification

The endocannabinoid system controls a variety of physiological and pathological states, such as immunomodulation, pain, addictive behavior, cognition, sociability, and stress response. Brain samples are obtained and subsequently analyzed for anandamide levels in the prefrontal cortex using liquid chromatography/tandem mass spectrometry (42). Monitoring anandamide levels has been used as a strategy in the treatment of depressive disorders. Anandamide is known to play a role in reducing neuroinflammation, a significant factor in depression. Microglial cells which act as phagocytes of the innate immune system in the CNS are the primary producers of anandamide (102).

Conclusion

This review has summarized several research methods employed to assess antidepressant activity in animals with a focus on depression triggers as well as behavioral and molecular changes. Among the methods discussed, CUMS is the most frequently used model to induce depression. CUMS is considered one of the best models to simulate human depressive states in animals as it is a naturalistic approach to expose animals to unpredictable stressors that disrupt homeostasis and cause somatic, physiological, neurobiological, and biochemical disorders and behaviors. Furthermore, FST is the most commonly used simple behavioral testing method which is consistent with antidepressant effects, reduces immobility time, and serves as the leading indicator of its effectiveness. Frequently observed molecular changes include those related to levels of monoamine neurotransmitters (5-HT, DA, and NE) and BDNF as major etiological contributors to depression. This review also reveals that the exploration of molecular changes in depression most often focuses on alterations in BDNF and monoamine levels. Increasing evidence suggests a correlation between BDNF function, monoamine levels, and the pathophysiology of depressive disorders. Therefore, these molecular changes are often observed in depression tests. These findings provide a valuable basis for future research on novel antidepressant treatments and the methods used to assess them.

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Authors' Contribution

Conceptualization: Ani Kristiyani, Zullies Ikawati.
Data curation: Ani Kristiyani, Zullies Ikawati
Formal analysis: All authors.
Funding acquisition: All authors.
Investigation: All authors.
Methodology: Ani Kristiyani, Zullies Ikawati
Project administration: Ani Kristiyani, Zullies Ikawati, Andayana Puspitasari Gani.

Resources: Ani Kristiyani, Zullies Ikawati, Andayana Puspitasari Gani.
Software: Ani Kristiyani, Zullies Ikawati.
Supervision: Zullies Ikawati.
Validation: Ani Kristiyani, Zullies Ikawati.
Visualization: Ani Kristiyani, Zullies Ikawati.
Writing–original draft: Ani Kristiyani, Zullies Ikawati.
Writing–review & editing: All authors.

Conflict of interests

The authors have declared that there are no competing interests.

Ethical considerations

The authors have completely observed ethical issues including double publication, plagiarism, and data fabrication.

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