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Animal model-based adjunctive herbal therapy in autism spectrum disorder: Therapeutic advances and prospects

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ABSTRACT

Autism spectrum disorder is a complex neurodevelopmental condition that poses major challenges to caregivers in contemporary societies. Since there is no established cure for this disorder so far, autistic patients of all ages are currently taken in charge of psychological and educational therapies that address their primary complaints while enhancing their quality of life. With the growing interest in herbal therapy globally, especially for illnesses with well-known underlying mechanisms, there have been clinical attempts to treat autism symptomatology using herbs or natural plant molecules in parallel with traditional follow-up. Nevertheless, basic research has only recently focused on studying the effects of herbal extracts in animal models of autism. This review emphasizes the animal studies that may provide credence to the adjunction of herbal therapy to conventional care strategies. Therefore, the study deduced a timeline chart combining promising herbal extracts such as *Camellia sinensis*, *Bacopa monniera*, and Korean red ginseng. This allows clinicians and caregivers to further evaluate the positive outcomes reported in autistic-like rodents. In conclusion, the study suggests employing herbal therapy as a clinical adjunct in the context of phytosupportive care.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition whose symptoms are noticeable in early postnatal life, affecting how children interact with their social environment. The global prevalence of this complex disorder is estimated to be 1% among children, but regional estimates vary substantially across countries depending on a panoply of genetic and environmental factors [1]. According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5-TR), autistic patients are diagnosed based on their difficulty communicating with people around them, their repetitive (stereotyped) behavior and restricted interests, and their inability to fulfill focus-demanding tasks at home and school. These clinical hallmarks of ASD are among the most difficult issues to deal with by both parents (or caregivers) and healthcare practitioners, thus posing a great socioeconomic burden worldwide [2].

Individuals with ASD are recognized to need particular care, compared to those with well-defined neuropsychiatric diseases, because the core communicative disabilities are mostly linked with a wide range of comorbidities, such as attention-deficit hyperactivity disorder (ADHD), anxiety, depression, irritability, and epilepsy [3]. Due to this multi-aspect condition, there is a consensus that ASD cannot be completely cured. Current treatments seek to attenuate symptoms while improving social interaction capabilities in autistic subjects of all age ranges. However, though these treatments are basically behavioral, cognitive, and/or physical, many autistic patients are prescribed medications to reduce anxiety, depression, and hyperactivity or to help

manage some difficult traits such as aggression and epileptic seizures [4]. Even though these approved drugs are effective in targeting each comorbidity apart, there is strong evidence that their use in the context of ASD may be negatively impactful in the long term, interfering with therapeutic efforts that aim at enhancing social abilities, mainly due to the poor understanding of pathophysiological mechanisms underlying ASD, which in turn makes difficult the process of predicting the outcomes in later life.

Psychotropic drugs used to treat mental health disorders, namely antidepressants, anti-anxiety medications, psychostimulants, antipsychotics, and mood stabilizers, are known for their acute and chronic toxicity, and their severe side effects can significantly alter the prognostic course in ASD patients, for whom physicians in charge should examine the best therapeutic option before a decision of giving them such drugs can be made [5]. Interestingly, there are important studies whose authors attempted to take advantage of herbs as a natural source of medications for treating various health conditions such as kidney injuries [6] and Alzheimer's disease [7], or even for low-toxicity contraception [8], reflecting that herbal therapy is emerging as an alternative to many synthetic drugs including psychotropic ones [9].

While not limited in practice, as about 80% of the world's population still relies on traditional medicines for their primary health care [10], herbal therapy for ASD is considered a recent approach, as shown by the relative novelty of studies. Its objective is to test plant extracts of sufficient efficacy and lower toxicity to be administered to autistic patients, alone or in conjunction with standard medical therapies. A larger consideration of herbal therapy that includes isolated plant molecules was applied in many experiments and trials on autism pathology; however, this usage does not reflect the whole benefit of medicinal herbs as usually accounted for in alternative medicine. The main candidate molecules that gave promising results are resveratrol [11] and sulforaphane [12], which are widely distributed in fruits, vegetables, and herbs and whose benefits are usually attained when eaten or decocted as a whole.

Therefore, the current report reviews animal studies relating to the use of plant extracts in autism therapy research and proposes a timeline chart for herbal therapy to aid in guiding future supportive care of autistic patients.

METHODS

In order to collect research articles relevant to herbal remedies applied to autistic patients and/or tested on animals made autistic-like experimentally, the authors conducted a literature search using the Mendeley reference management software and online system, targeting a variety of databases. The keywords utilized were "autism", "herbal", "therapy", "animal model", "autistic-like", "medicinal plant", "supportive care", and "alternative treatment". A reference script of relevant studies was obtained, organized, and then exported as a BibTeX file to the JabRef software that allowed the creation of a detailed HTML table file showing the author(s), title, abstract, year of publication, journal title, and DOI for each study. Further screening of the obtained studies prompted the authors to exclude those concerned with cannabis extracts and cannabinoids because the application of these treatments is beyond the scope of this review.

ADJUNCTIVE HERBAL THERAPY IN PRELIMINARY CLINICAL STUDIES

ASD is a complex brain dysfunction of developmental origin, meaning that many genetic and environmental factors combine *in utero* (and/or at the perinatal stage) to yield an unknown degree of damage to the brain structure and function, leading to a range of symptoms that generally appear in the first two years of life [13]. Clinically, this disorder is not looked at as a single disease but, rather, as a group of disabilities that assembles mild-to-severe communicative, behavioral, and intellectual abnormalities in the affected individuals, making almost every autistic person unique from a clinical point of view [14]. Therefore, although autistic patients share similarities, the clinical setting of follow-up and treatment can significantly differ between them, raising a major socioeconomic burden in modern societies. Patients with ASD are characterized by three coexisting neurobehavioral issues: deficient social communication and interaction skills, atypical repetitive behavior, and restricted and obsessive interests [15]. More or less related characteristics were also reported in the literature but are not specific to ASD, making the whole condition challenging for patients themselves and their caregivers (Figure 1).

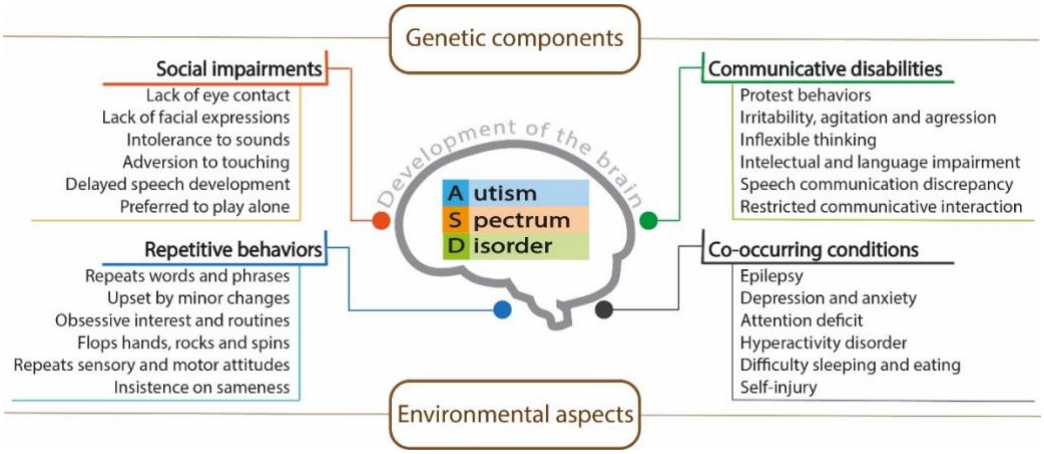

Figure 1. Overall signs and symptoms found in patients with ASD. This complex disease shares many signs and symptoms characteristic of other neuropsychiatric conditions, thus making both the diagnosis and subsequent treatment challenging for caregivers.

Three levels of severity are described in the DSM-5-TR, correlating with the increasing support that autistic patients need. However, there is no clear-cut picture of which therapies should be assigned to patients diagnosed with a certain level. Evidence-based educational and behavioral therapies are the most resorted to, attempting to effectively relay social information to ASD subjects, who are known to inadequately perceive and process social cues from their adjacent environment. Given the developmental character of this disorder, non-pharmacological approaches are meant to be applied to children but are frequently extended to be used in adolescents and adults. Table 1 summarizes the main conventional therapies and their most pertinent approaches that aim to reduce maladaptive behaviors while enhancing socialization and personal independence.

Table 1. Main conventional therapies and approaches catering to patients with ASD.

Therapy and corresponding approach(es)		Description	Ref.
Therapy	**Approach(es)**		
Applied Behavior Analysis (ABA)	Discrete Trial Training (DTT)	A teaching technique performed in a controlled environment, whereby skills desired to be learned by patients are broken into smaller behavioral steps. Repetition and reward are used to enhance learning of desired behaviors while ignoring those undesired or inappropriate in social situations.	[44]
	Pivotal Response Treatment (PRT)	A naturalistic teaching technique that takes place in everyday life contexts such as school and home. Using rewards, it aims to help patients learn some particular core behaviors, called pivotal skills, from which many secondary skills can easily be acquired.	[45]
	Incidental Teaching (IT)	A naturalistic teaching technique that takes place in everyday life contexts such as school and home. Unlike PRT, it uses patients' natural interests to promote the learning of desired skills. Leading their progress, patients are rewarded for appropriate behaviors they exhibit, reinforcing these behaviors to occur more often.	[46]
	Early Intensive Behavioral Intervention (EIBI)	A teaching technique with structured (methodical) instructions provided to patients at home or in a clinical setting. Relying on DTT, it anticipates desired behaviors by giving clear instructions (cues) that patients would respond to, replacing undesired attitudes (also called challenging behaviors) with new, positive skills.	[47]
	Positive Behavior Support (PBS)	A teaching technique qualified as person-centered or family-centered, whereby patients are urged to realize that all behavior serves a purpose to reach a particular want. In a comprehensive and rewarding manner, PBS helps patients set goals for themselves and come along, learning how to behave in particular, appropriate ways apart from challenging behaviors.	[48]
Comprehensive Treatment Models (CTM)	UCLA Young Autism Model	Also called the Lovaas Program, this approach relies on ABA therapy principles to teach positive skills by reinforcing smaller, more attainable behaviors. It is carried out on autistic preschoolers, who are systematically brought to feel social support every time they fulfill a new, appropriate skill. Through weekly planned sessions, undesired behaviors that impede learning are minimized.	[49]
	Early Start Denver Model (ESDM)	Designed for toddlers and preschool children, this play-based approach uses ABA to build interest in social communication through enjoying activities. The play environment creates motivation to keep communicating and self-expressing. Social skills are gradually acquired within this play context, whose activities are structured by therapists for intensive skill development.	[50]
	Treatment and Education of Autistic and Related Communication-handicapped Children (TEACCH)	A lifelong approach that covers the social development and personal independence of patients. It builds on innate strengths and skills to promote learning and communication through a structured, individualized schedule. This teaching approach puts major emphasis on organization and predictability of daily activities. A gradual transition from specialized centers to the family home is usually accomplished, where parents become the main contributors to therapy.	[51]
	Social Communication, Emotional Regulation, and Transactional Support (SCERTS)	This is a cooperative approach whereby parents, teachers, and therapists work together to help autistic children (preschool and primary school levels) learn how to control emotions while communicating with others. Relying on multiple techniques, the SCERTS program is not standardized but rather an individualized timeline of activities that best meets the needs of each child. It is, therefore, considered a supportive service in favor of families with autistic children.	[52]
Focused Interventions (FI)	Picture Exchange Communication System (PECS)	Autistic patients submitted to the PECS approach use cards with pictures, photographs, or symbols to express and obtain their wants. Acting as augmentative and alternative communication, PECS is implemented for patients with developmental delays including autistic ones, who may have difficulties with spoken language. Without verbal prompts, patients are taught to exchange on-card-drawn items for actual, desired objects (or actions). This way, communication skills are enhanced and progressively turned into simple, yet worthy, verbal requests.	[53]
Developmental therapy	Developmental, Individual-difference, Relationship-based model (DIR)	A large-scale approach, known as the Floortime Model, targets communicative, emotional, cognitive, and sensorimotor disabilities in all children diagnosed with developmental disorders, including ASD. Relying on both home and school contexts, DIR offers various activities of social interaction, such as daily playtime (usually on the floor) and problem-solving exercises, so that patients build new, adaptive skills to overcome challenges and reach personal milestones.	[54]
	Relationship Development Intervention (RDI)	This parent-led, individualized approach aims to help autistic patients use surrounding cues to think flexibly, understand social situations, and engage with people around them in an effective, appropriate manner. Attainment of these goals is expected to improve patient's quality of life, allowing them to handle the challenges of everyday life based on the sharing of ideas and feelings.	[55]
Occupational therapy	Sensory Integration Therapy (SIT)	Usually implemented as individualized program(s), SIT deals with the fact that autistic patients are mostly unable to combine information issued by the sense organs. As sensory integration disability prevents appropriate brain input of environmental and social cues, physical activities that involve more than one sense at a time are planned to teach the autistic brain to learn to process, summate, and regulate a mixture of sensory inputs, resulting in better emotional and social responses in front of real-world stimuli.	[56]
Cognitive Behavioral Therapy (CBT)	Functional Analysis of Behavior (FAB)	Commonly defined as one of the essential CBT steps rather than a stand-alone approach to it, FAB searches for reasons (called functions) behind maladaptive behaviors. Once undesired outcomes of such behaviors are first outlined and made clearer to patients, a series of observational analyses is performed backward along the behavioral chain to identify the root cause(s). The target behavior is, therefore, broken down into concatenated parts in search of those that must be changed. The ultimate goal is to alter the problematic thinking to achieve a new coping behavior with desired outcomes.	[57]
Speech and Language Therapy (SLT)	Speech and Language Therapy (SLT)	A specialized approach that improves verbal and non-verbal communication, with a particular focus on reciprocal social interaction. It is usually a group work therapy centered on speech deficits and related features such as semantic misunderstanding. Body language encoded in facial expressions and gestures is addressed meanwhile so that individualized SLT plans are prepared and tailored to the specific needs of each patient. As one of SLT goals, conversational skills are built upon the active contribution of parents and/or caregivers to therapy sessions.	[58]

Of interest, conventional therapies are not optional in the context of ASD but, rather, mandatory methodologies of chronic management of this lifelong condition. When necessary, pharmacological interventions are joined to the approach that appears to best fit the patient's needs. This is often the case because ASD subjects are prone to distress and irritability due to their inability to fulfill correct reciprocal conversations, thus feeling that their needs and expressions are neglected or misunderstood by family members and peers. Accordingly, anxiety, depression, and aggression are among the most frequent comorbidities found [16], prompting physicians to prescribe psychotropic drugs to help accomplish therapy goals, improve patients' quality of life, and minimize family suffering. In this respect, while the use of such drugs in neuropsychiatric diseases is regarded as primary care, many reports claim that their administration to ASD patients can negatively interfere with educational and behavioral efforts to alleviate the causal core symptoms, prejudicing the desired rehabilitation [17]. In addition, only two drugs have been approved by the Food and Drug Administration (FDA) for indication in autistic individuals, namely aripiprazole and risperidone, with the other psychoactive drugs being prescribed off-label to target certain symptoms due to the beneficial effects observed in clinical studies, thus limiting the range of medication choices and increasing the uncertainty associated with unapproved drugs in this context. Furthermore, there is currently no evidence regarding the efficacy of these drugs in ameliorating the core features of ASD. Yet, the overall toxicity and side effects of psychotropic drugs are widely reported [18], making it possible to exacerbate the autistic condition through somatic ailments. For these reasons, some clinical studies have been undertaken to administer plant extracts to autistic patients, medicated or not with an approved drug.

An adjunct therapy with *Ginkgo biloba* (EGb 761®, 100 mg twice a day) for at least 4 weeks was carried out on adult male patients (19.4-22.4 years old) already diagnosed with autistic disorder. Obvious improvement, though non-significant, was reported for scores relating to irritability, hyperactivity, inadequate eye contact, and inappropriate speech, suggesting a modest therapeutic effect [19]. In a similar study conducted by the same author, using *Hypericum perforatum* (commonly known as St. John's wort) as an adjunct treatment (20 mg daily), those autistic symptoms were significantly attenuated [20]. Moreover, when *Ginkgo biloba* (Ginko TD® tablets, 40 mg or 60 mg twice a day, depending on body weight) was administered daily to autistic children (4-10 years old) under risperidone treatment for 10 weeks, no significant behavioral changes were found in comparison to counterparts receiving risperidone alone, suggesting that the adjunction of this plant extract to risperidone did not affect the treatment outcome [21]. The effect of a 4-week adjunctive *Panax ginseng* treatment (pure extract tablets, 250 mg once a day) was also observed in three male autistic patients (18.4-22.2 years old) undergoing educational and behavioral interventions. This herb, which is comparable with the neuroprotective and anticonvulsant drug Piracetam [22], led to slight, non-significant improvement in the outcomes of interest (i.e., irritability, hyperactivity, inadequate eye contact, and inappropriate speech) [23]. In another study, capsules of sulforaphane-rich broccoli sprout extract were administered daily to young male ASD patients (13-27 years old) for 18 weeks without any psychotropic medication. The authors reported a substantial improvement of behavior in these patients, with a significantly greater number of them showing better social interaction and verbal communication compared to placebo-treated counterparts, proposing that this low-toxicity compound can be addressed for the prenatal prevention of ASD as well as for the early treatment of young children with this disorder [24]. Based on traditional Chinese medicine, clinical trials were also devoted to evaluating whether herbal preparations (as pills, capsules, or decoctions) help achieve better behavioral outcomes

in ASD patients. Overall, when such herbal medicines were combined with conventional therapy, most findings indicated a significant improvement in the assessment scale scores (reviewed in [25]). Taken together, these studies shed light on herbal therapy as a valuable supportive approach, making use of the beneficial effects herbs exert in various pathological conditions to treat ASD symptoms.

HERBAL THERAPY IN AUTISTIC-LIKE ANIMALS: A PHYTOSUPPORTIVE CARE

Animal models play a major role in understanding the etiological mechanisms of diseases and discovering new drugs to efficiently prevent or treat them. Prior clinical observations and findings allowed researchers to create autistic-like animal models that fulfill the main features of ASD, particularly the impaired sociability of the animal toward its conspecific mates. Rodents are particularly preferred because of their similarity to human core autism phenotypes and their high suitability for drug screening and preclinical trials [26]. Autistic-like rodents are generally produced by injection of chemicals, especially valproic acid (VPA), either to pregnant dams (i.e., prenatal approach) or to their newborn pups (i.e., postnatal approach). An off-gestational approach was also adopted, whereby induction of autism is performed in adult, non-pregnant animals. To our knowledge, only a few published studies explored the herbal extract effects in autistic animal models, meaning that herbal therapy in autism basic research is still in its embryonic stage but develops rapidly through clinical efforts to apply alternative medicines.

Camellia sinensis, commonly known as green tea, was among the first herbs applied in animal studies seeking anti-autistic remedies. Using postnatal induction of autism-like condition, through subcutaneous (SC) injection of VPA to newborn albino mice aged 14 days, Banji et al. [27] administered oral doses of *Camellia sinensis* extract (75 and 300 mg/kg/day) to both male and female offspring from postnatal day (PND) 13 to 40. This chronic treatment, particularly at the high dose, significantly attenuated the proprioceptive, motor, nociceptive, and spatial memory anomalies, thus preventing hyperactivity, high anxiety, and disorientation in a spatial recognition context. Evaluation of cerebellar areas in autistic-like animals further revealed damage to the Purkinje cell layer, which was mostly avoided by *Camellia sinensis* extract. It was concluded that this plant is capable of attenuating autism-related changes in motor coordination, cognition, and anxiety, thereby exerting a neuroprotective effect, which is partly explained by the antioxidant action revealed by the significant decrease in plasma malondialdehyde (MDA), a marker of lipid peroxidation. These preliminary findings were recently corroborated by another study centered on the neurochemistry of ASD. Male autistic-like rats were obtained through intraperitoneal (IP) administration of VPA to pregnant females on gestational day (GD) 12.5. Beginning on PND 15, oral treatment with *Camellia sinensis* extract (300 mg/kg/day) was provided for 20 days. Post-experiment neurochemical assays in untreated animals showed a significant decrease in the cerebellar and cerebral (cortical) levels of serotonin (5-HT), dopamine (DA), norepinephrine (NE), γ-aminobutyric acid (GABA), serine, and taurine, with a concomitant decrease in whole brain rates of cholesterol and antioxidant markers (GSH, SOD, and CAT). On the contrary, a significant increase in MAO, AChE, glutamate, aspartate, glycine, oxidative stress markers (MDA and NO), and proinflammatory cytokines (TNF-α and IL-6) was registered. These disturbances in VPA-exposed rats were significantly mitigated in those receiving *Camellia sinensis* extract, except for cortical 5-HT, cerebellar serine, and cortical/cerebellar NE, DA, glycine, and aspartate [28].

Bacopa monniera, a prominent Ayurvedic plant largely employed as neuroprotective, was also tested and retested in autistic-like animals. Intraperitoneal injection of VPA to pregnant rats (GD 12.5) led to offspring manifesting impaired olfactory discrimination on PND 9, delayed eye opening on PND 13 and 14, and impaired motor development on PND 8-12 as early signs of abnormal neurodevelopment. Upon weaning (PND 21), autistic-like male pups were divided into saline-treated and *Bacopa monniera* (L.)-treated groups. The aqueous extract of this plant, at a dose of 300 mg/kg/day, was administered orally from PND 21 to 35. When submitted to various tests during adolescence (PND 30-40) and adulthood (PND 90-110), autistic animals showed pain hyposensitivity, hyperactivity, low exploratory/social behaviors, and high anxiety attitudes. The authors reported cytoarchitectural alterations of the cerebellum along with a significant increase in hippocampal serotonin levels, among other neurochemical changes. Treatment with *Bacopa monniera* was found to significantly improve behavioral alterations, ameliorate neurochemical markers, and preserve cerebellum tissue integrity [29]. The neuroprotective features of *Bacopa monniera* were recently recalled through in-depth analyses. Using a standardized methanolic extract marketed under the name of BacoMind®, various aspects of the autism-like condition were targeted in male Wistar rats prenatally exposed to VPA (on GD 12.5). Developmental assessment of newborn rats on PND 7-23 revealed a delay in eye-opening, lower body weight, and deficient vestibule-related sensorimotor reflex in a tilting plane test. Hallmarks of neurobehavioral dysfunction determined between PND 44 and 53, were social deficits, anxiety-like and repetitive behaviors, learning and memory impairments, and poor motor coordination. Inherent in these visible disabilities are intense oxidative stress, proinflammatory cytokines rise (IL-1β, IL-6, and TNF-α), anti-inflammatory cytokines decline (IL-10), high neuronal injury scores, and glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor overexpression in the hippocampus and prefrontal cortex. Post-weaning oral treatment with the standardized extract (20, 40, and 80 mg/kg), which was carried on from PND 23 to 43, resulted in a significant improvement in almost all parameters at the dose of 80 mg/kg. It is worth noting that while a much lesser extent of prevention was registered at 40 mg/kg, both extract doses proved efficient when compared to risperidone as a reference drug, which failed to significantly relieve the autistic status [30].

The advantage of reference drugs in such experiments was revealed by another recent study about the anti-autistic potential of *Asparagus racemosus*. Intraperitoneal injection of VPA to pregnant Wistar rats (GD 13) was shown to produce metabolic and enzymatic disturbances in the brains of their offspring. Autistic symptoms were manifested as a decrease in body weight (PND 7, 14, 21, 28, and 35), delayed eye-opening (observed once daily), and impaired olfactory discrimination (PND 9). *Asparagus racemosus* extract (50, 100, and 200 mg/kg/day) was introduced upon weaning (PND 21-35) by oral route. This two-week treatment, only at 100 and 200 mg/kg/day, was significantly effective in ameliorating the high plasma nitrite levels, low brain GSH and catalase activity, and high monoamine oxidase A (MAO-A) and acetylcholinesterase (AchE) activities. The use of fluoxetine (selective serotonin reuptake inhibitor) and donepezil (acetylcholinesterase inhibitor) as reference drugs allowed to demonstrate that *Asparagus racemosus* extract at the higher dose (300 mg/kg/day) is powerful at preventing the biochemical deficits measured in autistic-like preadolescent animals [31].

Korean Red Ginseng (KRG), a popular herbal remedy in South Korea, is reportedly shown to enhance central nervous system (CNS) functions. Pregnant ICR mice, subcutaneously injected with VPA on GD 10, delivered male offspring with a socially impaired phenotype, as indicated by lower sociability indices and sniffing scores,

reflecting a poor social interaction of autistic-like animals with stranger mice. VPA-exposed animals also showed increased repetitive behavior and locomotor activity and decreased working memory scores and electroshock seizure threshold, while their motor coordination and balance measures did not differ from their control counterparts. These altered behavioral patterns, as assessed from PND 28 through 38, were normalized (except motor coordination ability) when autistic-like mice were given daily oral KRG solution (100 and 200 mg/kg) upon weaning (PND 21), revealing the potential therapeutic effects of KRG when administered post-weaning in animal models of ASD [32]. Using the same traditional remedy, Kim et al. [33] attempted to counteract VPA-induced autistic signs via concomitant administration of VPA and KRG during pregnancy. Pregnant Sprague-Dawley rats were subcutaneously injected with PVA on GD 12 while receiving daily oral doses of diluted KRG (20, 50, 100, and 200 mg/kg) from GD 10 to 15. Male autistic-like offspring showed hyperactivity (PND 28), social impairments (PND 30), and a lower electrical seizure threshold (PND 42) compared to their control counterparts. Poor social interaction was so evident that VPA-exposed pups significantly avoided conspecific strangers and preferred, thereafter, familial over novel rats. Depending on the dosage and variables measured, KRG treatment considerably improved the abnormal locomotor activity and sociability indices and prevented hypersensitivity to electric shock. Furthermore, KRG acted as an anti-teratogenic agent, counteracting the VPA-induced tail deformation in these animals. Those findings suggest that KRG may have positive therapeutic applications in ASD and serve as a preventive supplement against mild neural tube defects (NTD) in many contexts.

Oryza sativa, known within the Thai community as purple rice, was also explored for potential efficacy against autism symptoms. VPA was subcutaneously injected into newborn male and female rats on PND 14, followed by oral administration of the herbal extract (combined with pupae of the silkworm *Bombyx mori*) at doses of 50, 100, or 200 mg/kg between PND 14 and 40. A battery of repeated behavioral tests was applied throughout the treatment duration, with the cerebellum being isolated on PND 41 to assess biochemical markers and histomorphology. The extract was found to significantly reduce sensorimotor (reflex) deficits, anxiety-like behavior, social avoidance, and spatial memory deficiency shown in VPA-exposed offspring. However, this extract failed to counteract pain induced by thermal stimuli as a sign of nociceptive sensitivity following VPA exposure. Signs of severe oxidative stress, as well as a significant decrease in Purkinje cell density, were also unveiled in the cerebellum of autistic-like rats, whose treated counterparts had much less damage and even no apparent damage (at the dose of 200 mg/kg) at the end of the experiment [34].

Traditionally used for the treatment of many diseases, the leaf extract of *Morus alba* was recently shown to mitigate spatial memory deficit and hippocampal oxidative stress-related damage in Wistar rats. Subcutaneous VPA administration to 14-day-old male and female rats was later associated with pain hyposensitivity (PND 37-39), anxiety-like behavior, social avoidance, and spatial disorientation (PND 40). Neuronal density in hippocampal subregions (CA1, CA2, CA3, and dentate gyrus) was significantly decreased (PND 41), a finding that is linked with substantial oxidative stress. Applied by oral route (PND 14-40), daily treatment with *Morus alba* extract (25, 50, and 100 mg/kg) was unsuccessful in relieving social avoidance. While only low and medium doses differently affected nociceptive, oxidative, and cell density measures, all three doses resulted in a significant improvement in spatial memory performance [35].

Prangos ferulacea (L.) is a medicinal herb available in the Mediterranean and Middle East regions. Its various benefits in folk medicine prompted Saadat et al. [36] to evaluate its

efficiency in male Wistar rats made autistic by exposing them to VPA *in utero*. Dams receiving this drug on GD 12.5 (by IP route) were allowed to raise their pups until PND 30, from which *Prangos ferulacea* extract (100 and 200 mg/kg) was intraperitoneally administered to the male offspring until PND 58. Several behavioral tasks were used at the start and end points of treatment (PND 30 and 58), then the animals were euthanized on PND 65 for histopathological and biochemical assays in the brain. Signs of increased anxiety, decreased motor coordination, pain hypersensitivity, and hippocampal oxidative stress were found in the VPA-offspring-vehicle group compared to control groups. Interestingly, hippocampal levels of the apoptosis regulator proteins BAX and BCL-2 were significantly changed in autistic-like animals, with the BAX/BCL-2 ratio being increased, pointing to apoptotic damage within the brain. Indeed, the hippocampal CA1, CA3, and DG subregions manifested a high percentage of neuronal death. *Prangos ferulacea* extract showed mild anxiolytic and high antioxidant effects. With no significant change in BAX and BCL-2 levels, this extract largely prevented neuronal cell death in the hippocampus, particularly at the dose of 200 mg/kg.

Moreover, the hydroalcoholic extract of *Passiflora incarnata* was also explored for possible anti-autistic effects in a Wistar rat model. This plant extract, traditionally used to treat patients with anxiety and sleep disorders, was dissolved in drinking water and then given *ad libitum* to adolescent male offspring (PND 35-81) prenatally exposed to VPA. Untreated autistic-like rats showed anxiety-like and repetitive (stereotypic) behaviors, learning and recognition memory impairments, and low-score sociability measures. Serum oxidative stress markers were significantly changed, while damage to hippocampal CA1 and prefrontal neurons was considerable. *Passiflora incarnata* extract was found to alleviate some autistic-like behaviors, relating this finding to the neuroprotective and antioxidant action of its chemical constituents [37].

Postnatal induction of autism-like phenotype through administration of VPA to newborn mice was further performed by Tejano et al. [38], who evaluated the anti-autistic potential of the ethanolic leaf extract of Balakat tree (*Ziziphus talanai* (Blanco) Merr.). On PND 14-16, mice pups were orally treated with VPA syrup followed by the plant extract (300 and 400 mg/kg/day). Autistic-like pups submitted to motor behavioral tests and cerebellar histological analyses, meanwhile, exhibited a significant increase in geotactic latency scores and a reduction in Purkinje cell layer density, both of which are signs of inappropriate proprioceptive and motor processing. Post-VPA treatment with *Ziziphus talanai* extract was successful in keeping most behavioral scores at control-group levels while preserving the number and size of Purkinje cells in the cerebellar cortex, suggesting that *Ziziphus talanai* possesses ameliorative effects against behavioral aberrations and altered cerebellar histology in murine models of ASD.

Notably, in contrast to earlier moderate results reported in the clinical setting [19], daily treatment of newborn albino male mice with *Ginkgo biloba* extract (100 mg/kg, IP route, PND 13-40) was very favorable. Post-VPA (PND 14) behavioral, biochemical, histological, and immunohistochemical analyses provided insight into the complexity of ASD. Signs of high anxiety, poor social interaction with stranger conspecifics, and deficient working memory were observed in VPA-exposed animals not receiving the plant extract. This autistic-like condition was accompanied by significant neurochemical changes (increase in MDA, IL-6, and IL-17, and decrease in GSH and TGF-β1), damage in the Purkinje cell layer, and low expression of myelin basic protein (MBP) and serotonin in the molecular and granular layers of the cerebellum. The intensity of these neurobehavioral aberrations was significantly reduced by the chronic

Ginkgo biloba treatment, reflecting its therapeutic potential mainly through anti-inflammatory and antioxidant effects [39].

Interestingly, using an off-gestational approach whereby the chemical inducer propionic acid (PPA) was administered through intracerebroventricular (ICV) infusion, Jiji and Muralidharan [40] evaluated the ability of *Clitoria ternatea L.* root extract to counteract memory deficit and behavioral impairments seen in adult Wistar male rats (aged 4-8 weeks at the beginning of the study). Vehicle (1% Tween-80) or extract (250 and 500 mg/kg) solutions were provided orally for 28 consecutive days, with PPA being injected on days 22-28 in parallel to oral treatment. Altered learning and memory performances, along with a significant increase in serotonin and glutamate brain levels, were registered in PPA-exposed rats. Relating particularly to dampened object recognition and working memory, this animal model may mimic cognitive disabilities encountered in severe autism cases. Treatment with *Clitoria ternatea L.* extract significantly prevented the behavioral and neurochemical changes in a dose-dependent manner, highlighting that these significant nootropic effects are worthy of consideration in favor of patients with ASD.

Table 2 presents a summary underlining the main variables and findings of the reviewed animal studies. While we only mentioned positive results, it is noteworthy that half the reviewed studies enclose negative outcomes. *Camellia sinensis* did not alter certain neurotransmitters in the cerebral cortex (5-HT, aspartate, and glycine) and cerebellum (DA, NE, aspartate, glycine, and serine) [28]. *Bacopa monniera* failed to counteract thermal nociception in adolescent rats [29]. Treatment with Korean red ginseng did not reverse the impaired motor coordination and balance [32]. Treatment with *Oryza sativa* failed to significantly reduce thermal nociception and stereotypical behavior [34]. *Morus alba* was ineffective in improving anxiety, neuronal density, and SOD activity [35]. *Prangos ferulacea (L.)* did not affect the BCL-2/BAX apoptotic pathway, motor imbalance, anxiety, and aberrant responses to painful stimuli [36]. Moreover, treatment with *Ziziphus talanai* was unsuccessful in improving muscle strength in mouse pups [38]. Consequently, a more realistic and balanced view of the potential of herbal therapies for ASD is gained by taking into account the negative findings of the reviewed studies.

Table 2. Summary of the animal studies relating to using herbal extracts in autistic-like rodents.

Autistic-like models	Herb, dosage, and route of administration	Period of treatment	Main effects of herbal treatment*	Ref.
SC injection of VPA (400 mg/kg) to mouse pups on PND 14	*Camellia sinensis* at 75 and 300 mg/kg/day (PO)	PND 13-40	↓ Hyperactivity and anxiety-like behavior ↑ Sensorimotor reflex ↑ Spatial memory ↓ Plasma levels of MDA ↓ Cerebellar abnormalities (structural damage of Purkinje cells)	[27]
IP injection of VPA (600 mg/kg) to pregnant rats on GD 12.5	*Camellia sinensis* at 300 mg/kg/day (PO)	PND 15-34	↑ Cerebellar and cortical levels of GABA and taurine ↓ Cerebellar and cortical levels of glutamate ↑ Cortical levels of serine ↑ Brain levels of cholesterol, GSH, SOD, and CAT ↓ Brain levels of MAO, AChE, MDA, NO, TNF-α and IL-6	[28]
IP injection of VPA (600 mg/kg) to pregnant rats on GD 12.5	*Bacopa monniera* at 300 mg/kg/day (PO)	PND 21-35	↓ Hyperactivity and anxiety-like behavior ↑ Sensorimotor reflex ↑ Sociability ↓ Hippocampal levels of total nitrite ↑ Hippocampal levels of GSH and CAT ↓ Cerebellar abnormalities (low number and structural damage of Purkinje cells)	[29]

IP injection of VPA (600 mg/kg) to pregnant rats on GD 12.5	*Bacopa monniera* at 20, 40, and 80 mg/kg/day (PO)	PND 23-43	↑ Sociability and social preference ↓ Anxiety-like and repetitive behaviors ↑ Learning and memory ↑ Motor coordination ↑ Hippocampal and prefrontal GSH, SOD, CAT and IL-10 ↓ Hippocampal and prefrontal MDA, IL-1β, IL-6 and TNF-α ↓ Hippocampal and prefrontal expression of AMPA receptor ↓ Hippocampal and prefrontal abnormalities (structural damage of neuronal cells)	[30]
IP injection of VPA (500 mg/kg) to pregnant rats on GD 13	*Asparagus racemosus* at 50, 100, and 200 mg/kg/day (PO)	PND 21-35	↓ Plasma levels of nitrite ↑ Brain levels of GSH and CAT ↓ Brain levels of MAO-A and AchE	[31]
SC injection of VPA (300 mg/kg) to pregnant mice on GD 10	*Korean red ginseng* at 100 and 200 mg/kg/day (PO)	PND 21-38	↑ Sociability and social preference ↓ Repetitive behavior and hyperactivity ↑ Spatial working memory ↓ Hypersensitivity to electric seizure	[32]
SC injection of VPA (400 mg/kg) to pregnant rats on GD 12	*Korean red ginseng* at 20, 50, 100, and 200 mg/kg/day (PO)	GD 10-15	↑ Locomotor activity, sociability, and social preference ↓ Hypersensitivity to electric seizure ↓ Tail deformation (teratogenicity of VPA)	[33]
SC injection of VPA (400 mg/kg) to rat pups on PND 14	*Oryza sativa* at 50, 100, and 200 mg/kg/day (PO)	PND 14-40	↑ Sensorimotor reflex ↓ Anxiety-like and social avoidance behaviors ↑ Spatial memory ↑ Cerebellar levels of CAT, SOD, and GSH ↓ Cerebellar levels of MDA ↓ Cerebellar abnormalities (low density of Purkinje cells)	[34]
SC injection of VPA (400 mg/kg) to rat pups on PND 14	*Morus alba* at 25, 50, and 100 mg/kg/day (PO)	PND 14-40	↑ Sensorimotor reflex ↓ Social avoidance behavior ↑ Spatial memory ↓ Hippocampal levels of MDA ↑ Hippocampal levels of CAT and GPx ↓ Hippocampal abnormalities (low density of neuronal cells)	[35]
IP injection of VPA (600 mg/kg) to pregnant rats on GD 12.5	*Prangos ferulacea (L.)* at 100 and 200 mg/kg/day (IP)	PND 30-58	↓ Hippocampal levels of MDA ↑ Hippocampal levels of GSH and CAT ↓Hippocampal abnormalities (high neuronal death)	[36]
SC injection of VPA (600 mg/kg) to pregnant rats on GD 12.5	*Passiflora incarnata* at 30, 100, and 300 mg/kg in drinking water (PO)	PND 35-81	↑ Sociability and social preference ↓ Anxiety-like and repetitive behaviors ↑ Recognition memory ↑ Serum levels of CAT, SOD, and TAC ↓ Serum levels of MDA ↓ Cerebral abnormalities (high number of damaged neurons in the PFC and hippocampal CA1 areas)	[37]
IG gavage of VPA (400 mg/kg) to mouse pups on PND 14-16	*Ziziphus talanai (Blanco) Merr.* at 300 and 400 mg/kg/day (PO)	PND 14-16	↑ Locomotor behavior ↓ Cerebellar abnormalities (structural damage of Purkinje cells)	[38]
SC injection of VPA (400 mg/kg) to mouse pups on PND 14	*Ginkgo biloba* at 100 mg/kg/day (IP)	PND 13-40	↓ Serum levels of IL-17 ↓ Brain levels of MDA and IL-6 ↑ Brain levels of GSH and TGF-β1 ↑ Cerebellar levels of MBP and serotonin ↓ Cerebellar abnormalities (low number of Purkinje cells)	[39]
ICV infusion of PPA (4 µl of a 0.26 M solution) to male rats for 7 days (not specified)	*Clitoria ternatea L.* at 250 and 500 mg/kg/day (PO)	Not specified (treatment lasted for 28 days)	↑ Novel object recognition ↑ Learning and memory ↓ Brain levels of serotonin and glutamate	[40]

Relative to the autistic-like animals not given herbal treatment (↑, increase; ↓, decrease). Only statistically significant findings are mentioned. VPA, valproic acid; PPA, propionic acid; SC, subcutaneous; PO, per oral; PND, postnatal day; IP, intraperitoneal; GD, gestational day; IG, intragastric; ICV, intracerebroventricular.

PROSPECTS AND LIMITATIONS OF THE STUDY

Overall, the reviewed animal studies tried to replicate the complexities of human ASD by targeting the main pathophysiological factors thought to play a pivotal role in the occurrence and lifelong manifestations of this disease [41]. The autistic-like behaviors, which are relevant to most clinical observations in people with ASD, were associated with maternal use of medications during pregnancy (represented by valproic acid and propionic acid), as well as with neuroinflammation, brain oxidative stress, and neuronal cell damage at key cerebral regions in the offspring. Though describing a

chemically induced autism-like condition, these studies provide interesting findings indicating that herbal therapy merits consideration as an adjunct treatment to conventional therapies in patients with ASD. Table 3 summarizes the relevant mechanisms of action afforded by herbal extracts and the expected effects in patients with ASD.

To encourage the widespread use of herbal therapy in this context, we propose a timeline chart that relies on the animal developmental stage equivalent to a certain human age range (Figure 2). This equivalence, yet approximative, is based on studies that compare rodents' and humans' age at different phases of their lives [42]. As shown, herbal extract supplementation covers both prenatal and postnatal phases, with prenatal and/or perinatal utilization being considered preventive as no autism diagnostic procedures exist during pregnancy and childbirth. Caregivers should, therefore, rely on the medical history of pregnant women and any known risk factors to justify preventive herbal therapy. Accounting for the global use of herbal remedies and the fact that many herbal remedies used traditionally have become modern medicines through drug development [43], plant extracts reviewed herein are worthy of testing in ASD to aid in ameliorating psychological and educational program outcomes in persons already diagnosed.

Table 3. Protective mechanisms of herbal extracts in patients with ASD.

Herbal extract	Mechanisms of action	Expected effects	Ref.
Camellia sinensis	Upregulation of tyrosine hydroxylase and dopamine release in the hippocampus and striatum	Enhancement of learning and memory	[59]
Bacopa monniera	Upregulation of glutamate decarboxylase and enhancement of the $GABA_A$ receptor subunit synthesis in multiple brain regions	Reduction in hyperactivity, irritability, disorientation, anxiety, and depression	[60, 61]
	Inhibition of acetylcholinesterase and/or activation of choline acetyltransferase in forebrain regions	Enhancement of sociability	[62]
Asparagus racemosus	Inhibition of monoamine oxidase and acetylcholinesterase in the hippocampus and cortex.	Reduction in depression, learning impairments, and memory deficits	[63]
	Upregulation of BDNF synthesis and secretion in the hippocampus and frontal cortex	Enhancement of learning, memory, and sociability	[64]
Korean red ginseng	Upregulation of BDNF synthesis and secretion in multiple brain regions, particularly the amygdala and the frontal cortex	Enhancement of learning, memory, sociability, and motivational behavior	[65-67]
Oryza sativa	Upregulation of several hippocampal proteins, particularly the synaptic plasticity and neuronal trafficking markers α-synuclein and β-synuclein	Reinforcement of learning, memory, and adaptive behavior	[68]
Morus alba	Upregulation of GABA release in the whole brain	Reduction in hyperactivity, irritability, and repetitive behavior	[69, 70]
Prangos ferulacea (L.)	Modulation of cholinergic activity and blockade of L-type voltage-gated calcium channel in the gut	Enhancement of gut relaxation, eating behavior, and mood through the gut-brain axis	[71, 72]
Passiflora incarnata	Stimulation of $GABA_A$/benzodiazepine receptor	Reduction in hyperactivity, irritability, disorientation, anxiety, and depression	[73]
Ziziphus talanai (Blanco) Merr.	Preservation of Purkinje cells, granular layer, and molecular layer in the cerebellum	Enhancement of motor coordination and balance	[38]
Ginkgo biloba	Upregulation of dopamine and noradrenaline synthesis and release in the striatal and prefrontal regions	Enhancement of memory, mood, sociability, and motivational behavior	[74]
Clitoria ternatea L.	Down-regulation of acetylcholinesterase activity in the prefrontal cortex and hippocampus	Enhancement of learning, memory, and sociability	[75]

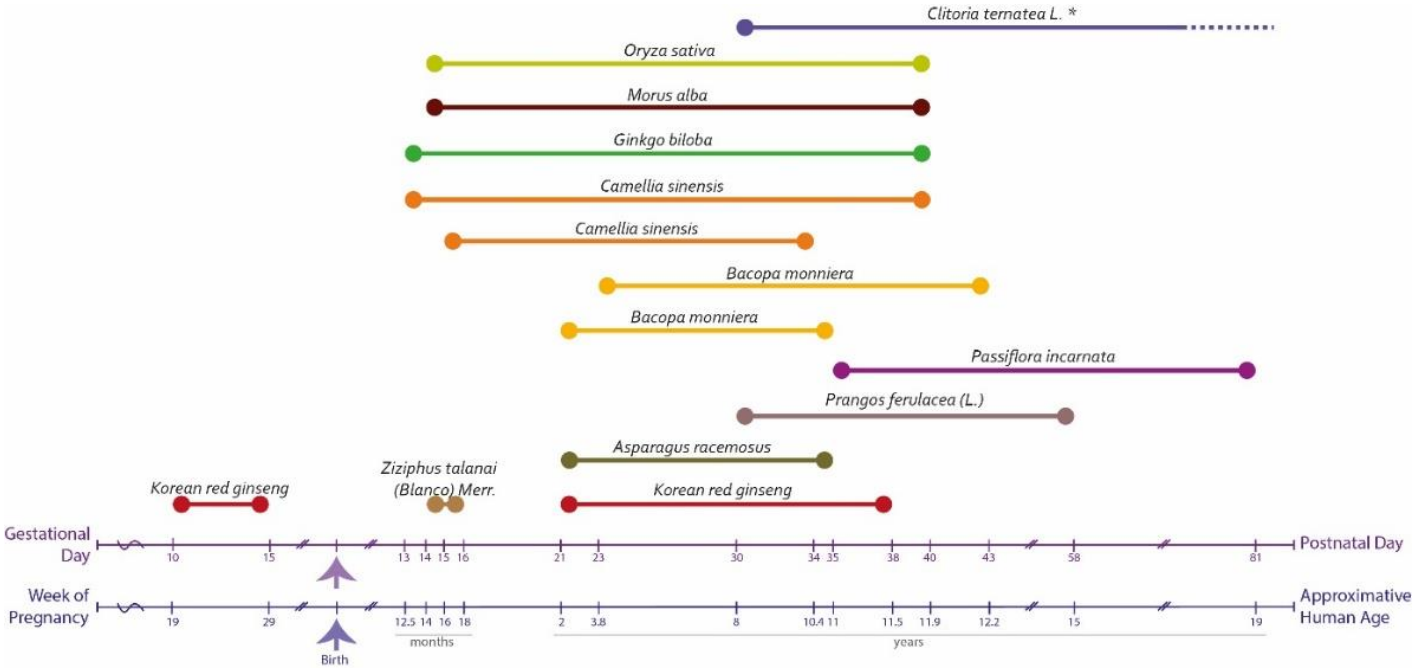

Figure 2. A timeline chart of adjunctive herbal therapies in patients with ASD. The time points indicated for each herb correspond to a certain human age, approximately equivalent to the animal's developmental stage. Herbal therapies are independent of each other, and their overlap in time does not signify herbal mixtures. * The dashed line indicates that the treatment endpoint was not specified in the corresponding animal study. The treatment with *Clitoria ternatea L.* begins at 4 to 8 weeks of age in animals, meaning that the minimum human age for administering this herb is approximately 6.8 years.

Nevertheless, the proposed timeline chart is not meant to be blindly applied to human subjects due to ethical considerations and toxicological measures regarding the dosage, route of administration, and duration of treatment. Rather, it can be referred to as setting appropriate clinical trials within the framework of ethical practice. The context of herbal therapy in ASD has, therefore, some limitations and practical issues that need to be thoroughly addressed in future research.

Certainly, the most significant issue to solve is the animal-human correlation in herbal efficacy. As stated above, herbal therapy in ASD is a recent approach worldwide, creating a big challenge to the extrapolation from rodents to humans. Even though the few clinical trials made so far are advantageous in this regard, considering prenatal and/or early postnatal phases as the main therapeutic window in most animal studies would add complexity to that challenge. Another limitation lies in the fact that herbal extracts are chemically heterogeneous, containing a wide range of active constituents that may interact with each other or with other metabolites inside the organism. While this property might allow patients to gain the whole benefit (in contrast to isolated molecules), heterogeneity means that even if improvement in autistic symptoms is observed, strict medical surveillance of patients under therapy would be mandatory to prevent potential side effects. Added to this is the likelihood that any prescribed herbal extract may alleviate some symptoms while exacerbating others. Thus, the appropriate use of herbal therapy for the sake of autistic people's well-being would remain speculative unless the extract heterogeneity is neatly explored to understand the corresponding pharmacokinetics and drug interactions. Furthermore, a substantial issue resides in the route of administration and doses applied, which are manifold among the reviewed animal studies. Accounting for the interspecies variation in toxicity, researchers should determine and put into practice a safety factor to achieve an acceptable oral dosage for humans. However, there are two studies [36, 39] whose

authors opted for the intraperitoneal route of administration, a choice that is inconsistent with the common human consumption of herbal extracts. So, it is highly desirable to reproduce the same experimental protocols using the oral route instead.

Otherwise, clinical attempts to implement herbal treatments for ASD should give particular attention to the poor communication skills of patients, who could be unable to express eventual discomfort (notably gastrointestinal). Their atypical behavior and sensitivity can make compliance with herbal ingestion a difficult task, particularly if the extract's taste or aroma is unpleasant. In these conditions, patient education is an integral part of herbal therapy, aiming at introducing it as a complementary and helpful care program that improves the quality of life.

On the way to overcoming these limitations, researchers should always bear in mind that autistic profiles are highly various, claiming the need for personalized schedules of herbal therapy in conjunction with conventional care. In this sense, biotechnology tools can be devoted to establishing patient clusters that share the same range of symptoms and severity, for whom standardized extract fractions fulfilling their therapeutic requirements are developed and manufactured. Refinement of the organoleptic properties of these extracts to facilitate ingestion and compliance should take place in the development of new biotechnological tools for ASD management.

CONCLUSIONS

Patients with ASD are currently handled by conventional therapies that aim to reduce their maladaptive behavior and enhance their communication skills. When needed, pharmacological interventions are called out by caregivers to control some comorbidities such as anxiety, depression, and aggressive behavior, but alternatives to psychoactive drugs are paving the way for herbal treatment of neuropsychiatric diseases. For instance, *Ginkgo biloba*, *Hypericum perforatum*, and *Panax ginseng* have shown promise in helping people with ASD. The emergence of animal studies focusing on the exploration of the anti-autistic effects of herbal extracts has further highlighted the forthcoming integration of herbal therapy into conventional healthcare programs, but this perspective presently confronts certain issues and limitations. In this review, we underlined the animal studies devoted to testing medicinal plants on autistic-like rodents, targeting the behavioral, histopathological, and molecular aspects of human ASD. A visual conclusion illustrating the key elements of this review is presented in Figure 3. To emphasize the developmental feature of these herbal remedy-based experiments, we deduced a timeline chart modeling the adjunctive herbal therapies in patients with ASD (Figure 2). Future research should be directed to analytical studies, in which predictive models of the relationships between herbal treatment and outcomes of interest are produced. Such statistical models would require a sufficiently large sample size, which is relatively small in the reviewed studies, and the comparison of herbal efficacy in males and females for better inference and precision. In this respect, extensive experiments covering the core symptoms and comorbidities of ASD should apply a variety of herbal fractions and therapeutic windows, allowing clinicians to systematically identify the best options for conducting cohort studies. Standardization of dosages and treatment durations among distinct experimental protocols is crucial in defining pharmacokinetics for clinical use. Taken together, both clinical and animal studies performed thus far should be endorsed, minimizing the time gap in the translation of their findings into approved herbal medications for patients with ASD.

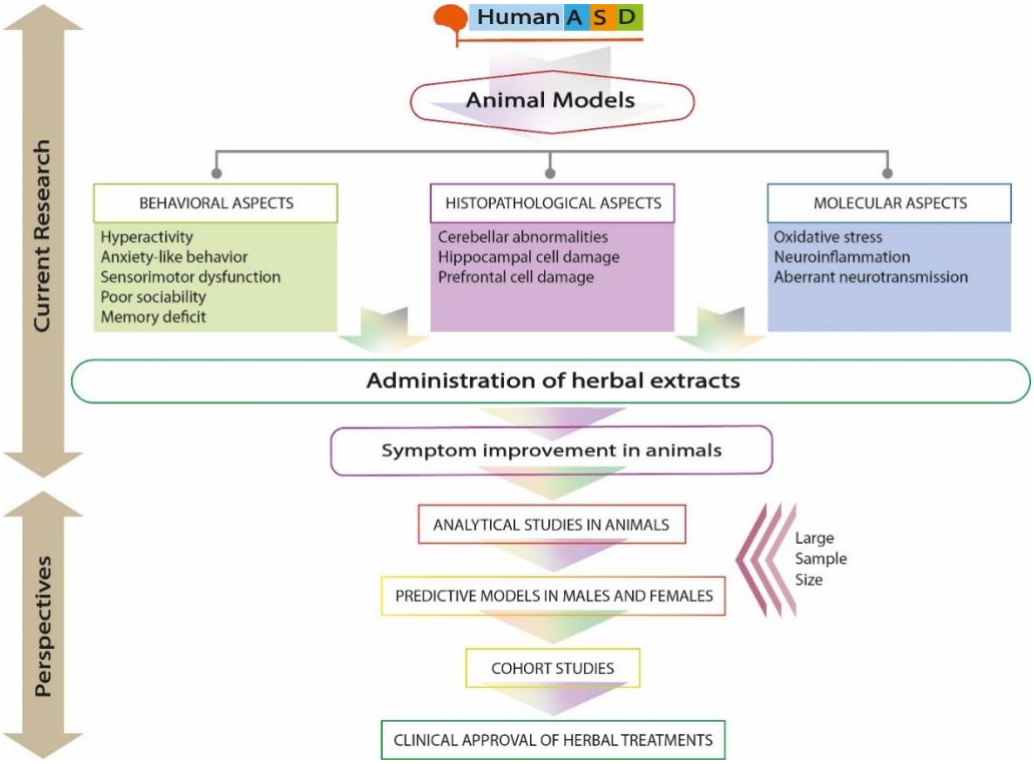

Figure 3. Current research relies on animal models that describe the behavioral, histopathological, and molecular aspects of ASD. In autistic-like rodents, herbal treatments have led to symptom improvement, thus opening the way for analytical studies that yield predictive models in males and females, accounting for a sufficiently large sample size. The established models would underlie the implementation of cohort studies and help ultimately approve herbal treatments for clinical use.

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AUTHOR CONTRIBUTIONS

MLT conceived the core idea, structured the review layout, performed the literature search and data extraction, and prepared the manuscript draft. SM prepared the manuscript draft, created the figures, and validated the final version. GS revised the draft and validated the final version. The authors read and approved the submitted version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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