Mood stabilisation with saffron and probiotic therapy

KEY LEARNINGS

Mental health disorders

- Depression is the most significant contributor to disability and affects more than 300 million people, representing approximately 4.4% of the world's total population.
- Depressive disorders are viewed as a collection of different symptom domains, including anxious and somatic symptoms, and as such, many individuals experience both depression and anxiety simultaneously.
- While frequently prescribed, recent reviews and meta-analyses have questioned the efficacy of standard antidepressant and anti-anxiety medications.

Pathophysiology

- The pathophysiology of mood disorders mainly involves four aspects: the dysfunction of the brain, the hypothalamus-pituitary-adrenal (HPA) axis, the immune system, and the gut-brain axis.
- While treatment options for depression and anxiety have primarily focused on pharmaceuticals that target monoamine neurotransmitters, serotonin, dopamine and norepinephrine, they are largely ineffective.
- Alternate options that address the complex biological pathways involved may provide more favourable outcomes for patients with mental health disorders.

Saffron

- Several underlying mechanisms of saffron in mood disorders have been identified, including its antioxidant, anti-inflammatory, serotoninergic and monoaminergic neurotransmission, neuroprotective and HPA-axis-modulating actions.
- Clinical trials suggest 30mg per day of saffron exerts superior effects in alleviating depressive symptoms compared with placebo and is as effective as antidepressants.
- Saffron has been clinically trialled for numerous conditions, including anxiety, stress, insomnia, premenstrual syndrome (PMS), sexual dysfunction, attention-deficit/hyperactivity disorder (ADHD), Alzheimer's disease (AD), ocular disorders, autoimmune diseases and metabolic conditions.

Cerebiome® (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175)

- The beneficial effects of probiotics on mood disorders may be explained by competitive exclusion of gut pathogens, reductions in pro-inflammatory cytokines, and communication with the central nervous system leading to changes in neurotransmitter function.
- Clinical trials involving Cerebiome® (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) have observed statistically significant effects on mood, anxiety, anhedonia, sleep quality, digestive disturbances and perceived stress in patients with depressive and stress-related disorders.

Mental health disorders

Mood disorders are one of the most prevalent psychiatric disorders and currently represent a leading cause of disability worldwide. Recent surveys indicate approximately 45% of Australians experience a mental disorder in their lifetime, with up to 21% reporting a mental illness in the past 12 months.[1] Data surrounding the current pandemic suggests a widespread change in the mental health status of the adult population. Approximately one-quarter of Australians reported mild-to-moderate symptoms of depression or anxiety during the early stages of COVID-19 restrictions, highlighting the need for better access to mental health care and early intervention.[2]

Depression is the most significant contributor to disability and affects more than 300 million people, representing approximately 4.4% of the world's total population.[3] Depressive disorders include two main

This technical sheet (V.05/22) is intended for the further education of practitioners. Any statements made do not intend to claim a cure for any condition mentioned.

sub-categories, including major depressive disorder (MDD) and dysthymia, and are characterised by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, tiredness and poor concentration.[3] Depressive disorders are increasingly viewed as a collection of different symptom domains, including depressive, anxious and somatic symptoms, with common underlying pathophysiological mechanisms.[4] As such, many individuals experience both depression and anxiety simultaneously.

Anxiety disorders are a major public health concern and include generalised anxiety disorder (GAD), specific and social phobias, post-traumatic stress disorder, obsessive-compulsive disorder (OCD) and panic disorder.[5] Anxiety is characterised by a number of mental and physical symptoms that may occur without any recognisable stimulus, occurring for at least six months.[6] Patients with anxiety and concomitant depression appear to have a lower response and remission rate, longer illness duration and greater disruption of social, work and family life than non-anxious depressed individuals.[7] Consequently, assessing the presence and severity of anxiety and monitoring response rates in patients with concomitant MDD is essential for positive clinical outcomes.

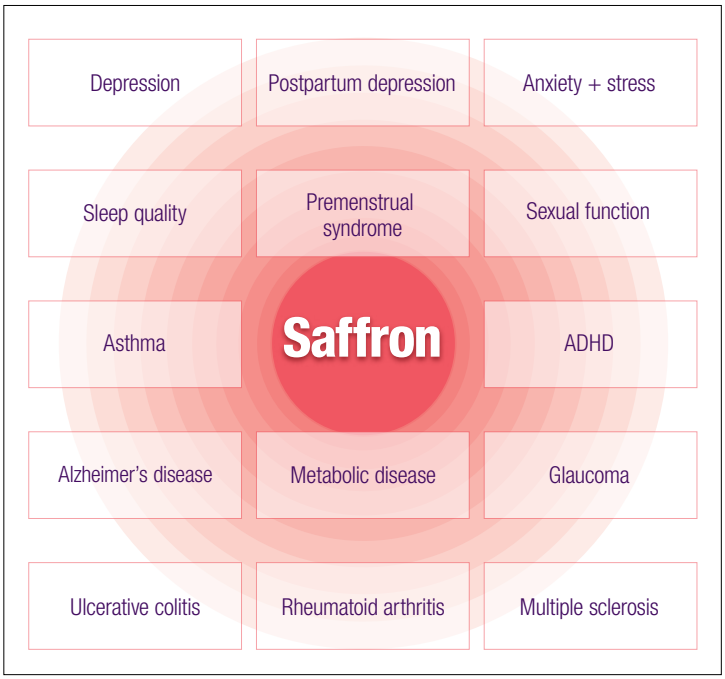
Treatment options for depression and anxiety have primarily focused on pharmaceuticals that target monoamine neurotransmitters, serotonin, dopamine and norepinephrine. While frequently prescribed, recent reviews and meta-analyses have questioned their efficacy.[8-10] Only one out of nine people may benefit from antidepressants, while the remaining eight individuals are unnecessarily put at risk of drug side effects,[10,11] such as suicide attempts, cardiovascular events, and severe withdrawals after discontinuation.[12] Moreover, current anti-anxiety medications have insufficient overall short- and long-term efficacy and possess adverse effects when prescribed for long periods.[13]

The pathophysiology of mood disorders mainly involves four aspects: the dysfunction of the brain, the HPA-axis, the immune system, and the gut-brain axis. Brain abnormalities are mainly reflected by neurotransmitter dysregulation and impaired neuroplasticity.[14] HPA-axis imbalance manifests by dysregulated negative feedback mechanisms and reduced brain-derived neurotrophic factor (BDNF), serotonin synthesis and glutamate receptor expression.[15,16] Sustained activation of the immune system is associated with inflammation and the release of chemokines and cytokines,[17] further inhibiting the negative feedback of the HPA-axis and disturbing neurotransmitter production.[18,19] The gastrointestinal microbiota is a crucial component of the gut-brain axis that affects the development of the immune system, influences the regulation of neurotransmitters, affects neurogenesis and can impact the maturation of the HPA-axis.[20] Given the ineffective pharmacological options and the complex biological pathways involved, alternate therapeutic options that address disturbances in the HPA-axis, neuroinflammation and microbiome development may provide more favourable outcomes in patients with mental health disorders.

Saffron

Saffron is the dried stigma of the *Crocus sativa* flower and has a long history of use as a spice, colouring agent and medicine.[21] Native to the Middle East and countries of the Mediterranean, up to 80% of annual saffron harvest originates in Iran.[22] With three stigmas to every flower, approximately 200,000 flowers must be harvested to produce one kilogram of dried saffron and thus, it is considered to be the most expensive spice in the world. Consequently, adulteration is frequent, with up to 12% of saffron samples failing quality specifications.[23] Ensuring authentic material is therefore critical in terms of efficacy and patient safety.

Figure 1: Clinical indications for saffron



safr'inside™ THINK POSITIVE

Safr'Inside™ is a high quality, clinically trialled saffron extract, containing at least 2% safranal and 3% crocins.

Apart from its traditional value, saffron has gained increasing awareness for supporting numerous diseases, including asthma, liver disease, cramps, menstrual disorders, pain and cancer.[24] The medicinal properties are likely due to a number of constituents found within saffron, including crocetin, crocin and safranal, which have been found to have potent antioxidant, anti-inflammatory, antidepressant, antitumour, anticonvulsant and anti-hyperlipidaemic effects.[25] Additionally, saffron contains over 150 volatile and non-volatile active compounds, many of which are carotenoids such as zeaxanthin, lycopene, beta-carotene and polysaccharides. Given the many actives in saffron, several underlying mechanisms relevant to treating mood-related disorders include its antioxidant, anti-inflammatory, serotoninergic, monoaminergic, neuroprotective and HPA-axis modulating effects.[26]

SAFFRON MECHANISM OF ACTION	
Anti-inflammatory	Crocin exhibits dual inhibitory effects against cyclooxygenase-1 (COX 1) and cyclooxygenase-2 (COX-2) and reduces the production of prostaglandins.[27] *In vivo* studies demonstrated inhibition of TNF-α, IL-1, IL-6, nuclear factor-κB (NFκB), interferon-γ (INF-γ) and inducible nitric oxide synthase (iNOS).[28,29]
Antioxidant	Crocetin and safranal have been shown to re-establish the antioxidant status of glutathione peroxidase, superoxide dismutase and catalase, and reduce lipid peroxidation.[28,30] While each constituent has strong antioxidant effects, its potency is greatest when delivered synergistically.[31]
Serotoninergic and monoaminergic	Crocin and safranal inhibit the reuptake of dopamine and norepinephrine and exhibit antagonistic action at 5-HT receptor sites.[32-34] Safranal shows anti-anxiety activity by potentially binding to the benzodiazepine site at GABA-A receptors.[35] Crocin has a weak but significant affinity to the N-methyl-D-aspartate (NMDA) receptors *in vitro*.[24]
HPA-modulating	Saffron has also been shown to reduce plasma levels of corticosterone in response to stress in both acute and chronic rodent studies,[25,36] suggesting potential modulation of the HPA-axis.
Neuroprotective	Crocin increases levels of BDNF and elevates concentrations of VGF (a neuropeptide) in the hippocampus of mice.[37] Pre-treatment with safranal also protects the hippocampus against oxidative stress by lowering lipid peroxidation levels and inhibiting DNA damage.[38]

Clinical indications

Depression

In recent years, increasing attention has focused on the antidepressant activity of saffron in treating mood disorders. Although not completely understood, the effects of saffron on mental health may be the result of a synergistic function of several constituents, primarily crocin and safranal.[35] Saffron may inhibit the reuptake of monoamine neurotransmitters in the brain and reduce pro-inflammatory cytokines, which all serve as mediators in environmental and genetic factors that contribute to the development of depression.[39] Implicated in the aetiology of mood disorders, increased oxidative stress also accelerates inflammatory and immune responses and disrupts neurotransmitter balance, further influencing the development of depression.[40-42] Given the known properties of saffron, the beneficial effects observed on mental health parameters may similarly be attributed to its ability to re-establish the antioxidant status and defend cells against oxidative stress.

In the last two decades, nine meta-analyses have demonstrated the efficacy of saffron in the treatment of adults diagnosed with MDD, mild-to-moderate depression and low mood with various control groups.[21,26,43-49] Several studies have found saffron at doses between 30mg and 60mg to be equally effective as the antidepressants fluoxetine, imipramine, sertraline and citalopram at improving depressive symptoms, with generally fewer reported adverse effects.[7,50-55] Saffron has shown to have large treatment effects compared to placebo in patients aged over 18 years with MDD, mild-to-moderate depression or depressive symptoms, with significant improvements observed in Beck Depression Inventory (BDI) Scores and Hamilton Depression Rating Scales (HDRS-DA).[56-61] These positive results have been observed across various population groups, including the elderly,[55] post-menopausal women experiencing hot flushes[56] and patients with comorbidities such as metabolic syndrome (MetS)[60] and HIV.[59]

Overall, the cumulative results confirm that saffron exerts superior effects in alleviating depressive symptoms compared with placebo and is as effective as antidepressants in managing this debilitating disorder. For the majority of studies, saffron at 30mg per day for up to 12 weeks appears safe and is well-tolerated. Interestingly, lower premorbid antioxidant levels and higher inflammatory states are associated with an increased rate of non-responders from antidepressant treatments;[62,63] therefore, saffron as an adjuvant agent may be particularly relevant.

Anxiety and stress

Neurobiological factors and disturbances in neurotransmitters are thought to be potential causes in the development of GAD and other anxiety disorders. Considered a major predisposing factor in anxiety, chronic stress also significantly impacts the homeostatic biological system by increasing the allostatic load. The precise mechanism by which saffron produces anti-anxiety effects remains under investigation; however, it has been demonstrated that certain constituents display an affinity for the benzodiazepine binding site at the GABA-A receptor.[5] According to animal studies, crocin may additionally interact with the HPA-axis and reduce stress-induced corticosterone increase,[64] inhibit NMDA opioid receptors,[65] and enhance hippocampal BDNF levels.[66] Given the previously reported benefits in mood disorders, multiple studies have assessed the efficacy of saffron in comorbid depression and anxiety, GAD, and stress.

The results of five clinical trials assessing the effects of saffron for the treatment of anxiety accompanied by depressive disorders have conclusively demonstrated significant improvements in symptoms across various population groups.[7,67-70] Most significantly, saffron at 30mg per day for six weeks was as effective as citalopram in improving symptoms of anxiety and depression in patients with MDD and anxious distress.[7] Based on self-reports, saffron was also associated with greater improvements in overall internalising

symptoms, separation anxiety and social phobia in youths with mild-to-moderate anxiety and depression.[70]

Saffron as an adjuvant with sertraline significantly reduced the mean Hamilton Anxiety Rating Scale (HAM-A) compared to placebo in patients diagnosed with GAD for six weeks ($p = 0.029$).[6] A recent study exploring the effects of 30mg per day of saffron on emotional wellbeing found a beneficial effect on heart rate variability in response to psychosocial stressors.[67] These findings suggest saffron may be particularly relevant for increasing resilience against the development of stress-related disorders. Interestingly, saffron at 25mg was also found to be more effective than diazepam at lessening trait anxiety perioperatively in 102 patients ($p = 0.019$).[71]

Postpartum depression

Postpartum depression (PPD) has a global prevalence of up to 82% and often occurs within two to four weeks after delivery.[72] This condition has major implications for the mother's ability to function and care for her baby and the infants' developmental and health outcomes.[73] While not entirely recognised, genetic factors along with neuroendocrine, neurotransmitter and neuroinflammatory changes are associated with the underlying neurobiology of PPD.[74] Management frequently includes antidepressants; however, they are often associated with unwanted side effects, and many new mothers cannot tolerate or do not respond effectively to these treatments. Furthermore, as antidepressants may be excreted through the breastmilk, breastfeeding mothers are often reluctant to take these medications over concerns about the potential effects on their infant.[75] Complementary approaches deserve far greater attention given the high prevalence of PPD and the limited effective pharmaceutical treatments available.

Two clinical studies have assessed the efficacy of saffron in the management of PPD. In a multi-centre, double-blind, randomised clinical trial, 64 patients with mild-to-moderate PPD were randomised to receive saffron (15mg, twice daily) or fluoxetine (20mg, twice daily) for six weeks.[76] There was no significant difference between the two groups regarding reducing the Hamilton Depression Rating Scale (HDRS) scores ($p = 0.61$), and remission rates were almost equal between the two groups. In the saffron group, 40.60% of patients experienced a complete response, compared to 50% in the fluoxetine group; however, the in-between difference was not statistically significant. Patients in the fluoxetine group experienced more adverse effects than the saffron group, including headache, dry mouth, daytime drowsiness, constipation and sweating.

In a double-blind, randomised and placebo-controlled trial, saffron (15mg, twice daily) had a more significant effect on the Beck Depression Inventory-Second Edition Score than placebo in 60 new mothers suffering from mild-to-moderate PPD.[73] In the final assessment, 96% of patients in the saffron group were in remission compared to 43% in the placebo group ($p < 0.01$). Moreover, only 6% of patients had complete response rates in the placebo group, compared to 66% in the saffron group. No significant adverse effects were documented in this study, implying that 30mg of saffron can be used safely by breastfeeding mothers. The available evidence would suggest that saffron is a safe alternative to antidepressants in managing PPD and may reduce symptoms of this debilitating condition.

Sleep quality

Up to 45% of adults in Australia report difficulty falling or staying asleep, which has significant health implications on both physical and mental wellbeing.[77] Poor sleep has been associated with a 2.6 times greater risk of developing depression,[78] and both anxiety and depression have an indirect relationship with the degree of insomnia severity.[79] Since saffron has demonstrated positive effects on depression and anxiety symptoms, researchers have investigated whether the sleep-enhancing effects of saffron may also result from its impact on affective disorders.

Multiple studies have observed significant sleep quality, latency, and duration improvements after saffron supplementation (28-300mg daily).[80-84] These improvements have been observed across various populations, including patients with anxiety, depression, type 2 diabetes (T2D) and obesity.[69,84] While the mechanisms associated with saffron's potential sleep-enhancing effects are uncertain, its active constituents are known to modulate neurotransmitter levels implicated in sleep and insomnia, such as serotonin, dopamine, norepinephrine, and GABA.[32, 85] Crocin was also suggested to modulate the histaminergic arousal system to induce non-REM sleep in mice.[86] Given insomnia is associated with increased inflammatory markers,[87] saffron's anti-inflammatory properties may similarly be associated with its restorative sleep effects.

Other indications

Premenstrual syndrome

PMS affects up to 40% of women and is characterised by moderate psychological and physical symptoms during the late luteal phase.[88] Dysregulation of the serotonergic system and the impact of sex hormones on serotonin uptake and transport has been associated with the majority of PMS symptoms, including depression and behavioural changes.[89,90] Two clinical trials have found saffron (30mg daily) significantly improves PMS related symptoms compared to placebo in women with PMS.[91,92] Saffron was also found to be comparable to fluoxetine in reducing abdominal bloating, depression and anxiety in 164 women suffering with PMS ($p > 0.05$).[93] The authors proposed that the reductions in pain may relate to the antioxidant properties of saffron via reducing the production of free radicals and prostaglandins.

Sexual function

Selective serotonin reuptake inhibitor (SSRI)-induced sexual dysfunction has been reported to affect up to 70% of individuals and is one of the most common causes of drug discontinuation.[94] Traditionally recommended as an aphrodisiac agent, saffron has been shown to effectively improve certain SSRI-induced sexual problems in male and female patients with major depression.[95,96] Erectile function, intercourse satisfaction and total scores were significantly improved in male participants, while improvements in total Female Sexual Function Index Score, arousal, lubrication and pain were observed in

women. Saffron was also associated with significant improvements in erectile and orgasmic function, sexual desire, and intercourse satisfaction in men with erectile dysfunction (ED) after 10 days.[97] While the mechanism is not entirely known, saffron appears to interact with several neurotransmitters that may play a role in sexual reflexes and erectile function.[98,99] Furthermore, the protective effects of saffron on ischemia-induced oxidative stress may be, in part, responsible for the benefits observed in ED.[100]

Attention-deficit/hyperactivity disorder

ADHD is a heterogeneous condition, with the majority of patients experiencing comorbid or associated psychiatric disorders, such as anxiety, tic disorders, OCD, bipolar disorder, and specific learning disorder.[101] While methylphenidate (Ritalin) is the most commonly prescribed drug for ADHD and improves cognition via a dopaminergic effect, it is associated with a variety of side effects, and up to 30% of children are non-responders.[102] Saffron has been shown to be as effective as methylphenidate, per improvements observed in the Parent and Teacher Rating Scale scores in a randomised, double-blind clinical trial.[103] In an additional study, saffron in combination with methylphenidate was more effective than methylphenidate alone in lowering the ADHD Rating Scale-IV in ADHD school children ($p < 0.05$).[104] Since constituents found in saffron have been shown to inhibit dopamine and norepinephrine reuptake at the synapse[105] and are NMDA receptor antagonists,[106] it could be hypothesised that saffron benefits ADHD patients owing to these positive effects on monoaminergic and glutamatergic systems.

Alzheimer's disease

AD is an irreversible, progressive neurodegenerative disorder that leads to severe social and economic impacts on the healthcare system.[107] Memantine, an NMDA receptor agonist, and donepezil, an acetylcholinesterase inhibitor, are widely accepted agents to attenuate certain AD-related symptoms; however, unfavourable side effects and lack of optimal efficacy has forced researchers to investigate alternative strategies.[108] One clinical study observed significantly greater improvements in cognitive scales in patients receiving saffron compared to placebo,[109] while two subsequent trials found saffron to be as effective as donepezil[110] and memantine[111] in improving cognition and reducing cognitive decline, with lower adverse effects. Among the potential mechanisms, saffron's benefits in memory disorders may be attributed to its antioxidant capabilities.[112] Saffron has also demonstrated inhibition of beta-amyloid (Aβ) fibril formation and destabilised pre-formed Aβ fibrils *in vitro*.[113] Saffron also decreased glutamate levels, exerted antagonist effects on NMDA receptors, and inhibited acetylcholinesterase activity in animal and *in vitro* studies.[114-116]

Metabolic diseases

It has been well established that inflammation and oxidation within adipose tissue instigates systemic insulin resistance and chronic inflammation,[117,118] often resulting in a broad range of metabolic diseases, including MetS, T2D and non-alcoholic fatty liver disease (NAFLD). A growing body of evidence suggests that saffron reduces the production of IL-6 and TNF-α and suppresses the activation of inflammatory signalling during high glucose conditions, which play a vital role in insulin resistance and the development of metabolic conditions. In various clinical trials, saffron has been shown to decrease fasting blood sugar, cholesterol, triglycerides and HbA1c levels in prediabetic,[119] type 2 diabetic[120,121] and MetS patients.[118] A significant trend toward increased antioxidant markers and reduced inflammatory cytokines was also observed after saffron supplementation in patients with MetS,[118] T2D[121] and NAFLD.[122]

Macular degeneration and glaucoma

Age-related macular degeneration (AMD) and glaucoma are the most common causes of permanent visual loss in the elderly population. Saffron has generated interest in ocular diseases, putatively attributable to its antioxidant properties. Moreover, crocin and crocetin have been shown to suppress pro-inflammatory pathways, such as NFκB,[123] enhance oxygen diffusion[124] and improve ocular blood flow,[125] factors that play an essential role in disease development. Based on the results of six published clinical trials involving AMD patients, objective and subjective measures of visual acuity were significantly improved after saffron supplementation at doses between 20mg and 50mg, even with relatively short-term durations.[126-131] In an additional study involving glaucoma patients, 30mg of saffron alongside the topical application of timolol and dorzolamide resulted in a significant reduction in intraocular pressure after three weeks.[132] Improvements in the overall inflammatory and oxidative stress load positively impact visual parameters, suggesting saffron could be considerd an effective and safe adjunct in AMD and glaucoma management.

Autoimmune diseases

Excessive oxidative stress has been associated with the pathogenesis of autoimmune diseases (AIDs) by increasing inflammatory mediators, disturbing immunological tolerance and inducing apoptotic cell death.[133] It has been reported that saffron modulates signalling pathways, such as NFκB, and controls the expression of genes encoding inflammatory cytokines, adhesion molecules, inducible enzymes and chemokines, many of which play a significant role in regulating the immune system.[134] A clinical trial has demonstrated significantly reduced disease severity and enhanced antioxidant status after saffron supplementation in 75 patients with ulcerative colitis.[135] An additional study found saffron to significantly reduce tender and swollen joints, pain intensity and disease activity score in 61 women with rheumatoid arthritis.[136] In patients with relapsing-remitting multiple sclerosis, high doses of saffron also significantly reduced serum levels of MMP-9, an enzyme that is associated with disease pathogenesis.[137]

Asthma

Asthma is defined by respiratory symptoms that vary over time and intensity and is usually associated with chronic airway inflammation.[138] Saffron may benefit people living with asthma by inhibiting histamine H1 receptors, muscarinic receptors and calcium channels, modulating nitric oxide production, and activating β2-adrenoceptors.[139] In two clinical studies, saffron supplementation effectively alleviated the

frequency of clinical pulmonary symptoms in patients with allergic asthma.[138,140] Significant reductions in anti-heat shock protein 70 (a risk factor for asthma severity), eosinophils and basophil concentration, and high sensitive-C reactive protein was also observed, highlighting the anti-inflammatory and immune-modulating activity of saffron.

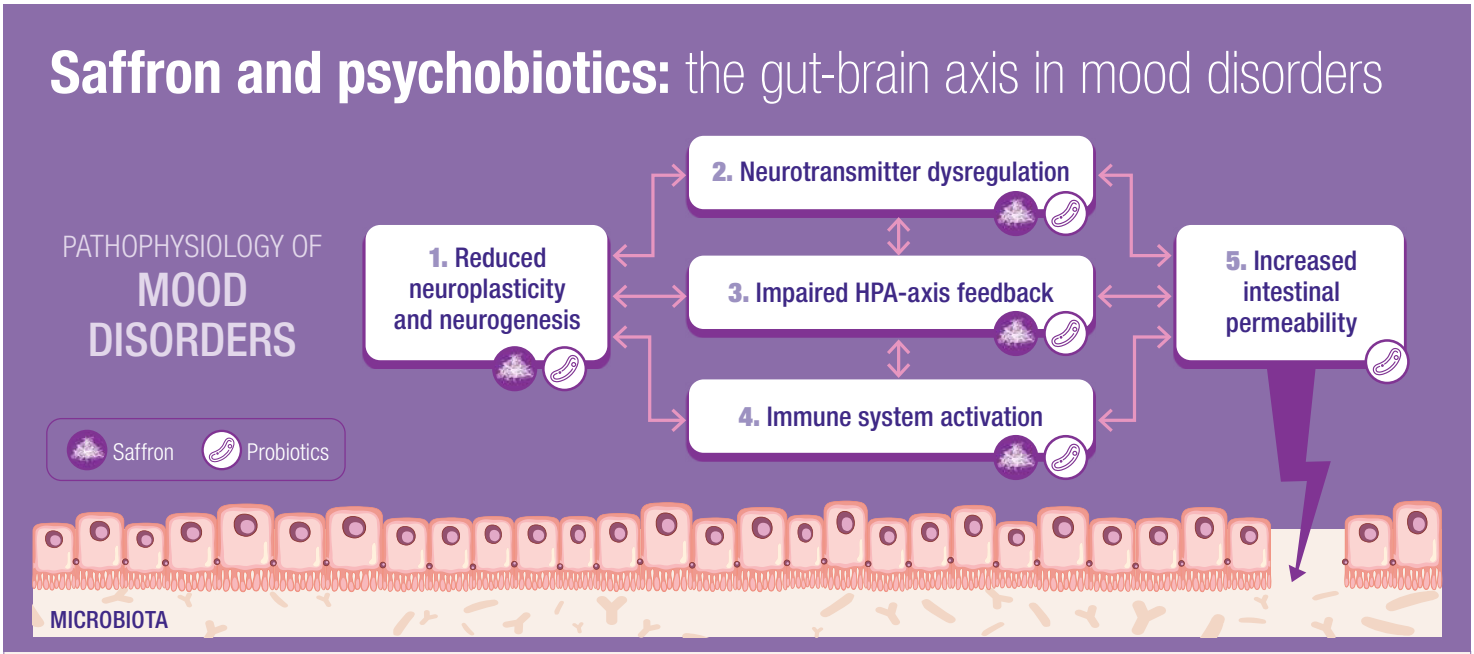
The Gut-Brain Axis in Mood Disorders

Biochemical signalling between the gastrointestinal tract and the central nervous system, otherwise known as the gut-brain axis, occurs via the autonomic nervous system, enteric nervous system, neuroendocrine, and immune systems.[141] Significant progress has been made in recognising the importance of this bi-directional relationship with researchers exploring how the microbiota influences mood disorders. Key findings show that gastrointestinal bacteria directly synthesise neurotransmitters, such as serotonin, GABA, histamine, adrenaline, and other neurotrophic factors, including BDNF.[142] Stress is known to increase intestinal permeability, which allows gram-negative bacteria to cross the mucosal lining, leading to the activation of the immune system and the subsequent production of inflammatory mediators.[143] Moreover, alterations in the microbiota have been associated with hyperactivity of the HPA-axis, resulting in altered behaviour.[142] Recent advances have linked nervous system disorders to changes in the microbiome, further emphasised by the high rate of comorbidity between psychiatric and gastrointestinal disorders.[144] As such, preclinical and preliminary data suggests modulating the microbiota via probiotic supplementation in psychiatric disorders paves the way for more novel treatment options.[146]

Cerebiome® (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175)

The beneficial effects of probiotics on mood disorders may be explained by competitive exclusion of gut pathogens, reductions in pro-inflammatory cytokines, and communication with the central nervous system leading to changes in neurotransmitter function.[146] However, not every probiotic species is beneficial in all circumstances, and careful selection of appropriate strains based on preferred clinical outcomes is of great importance.[147]

Lactobacillus and *Bifidobacterium* are the main genera of microorganisms, with several studies demonstrating their beneficial effects on host behaviour. *Lactobacillus helveticus* R0052 was originally isolated from dairy culture and it has been characterised for its ability to adhere to human intestinal epithelial cells, block the invasion and translocation of pathogenic bacteria and down-regulate pro-inflammatory pathways.[148] Known as a fermentative bacteria, *Bifidobacterium longum* R0175 has been shown to maintain intestinal barrier integrity, interfere with the inflammatory response and may



CEREBIOME® MECHANISM OF ACTION	
Preserves neuroplasticity and neurogenesis	Attenuates the decremental effects of lipopolysaccharide (LPS) on memory through BDNF expression, suggesting improvements in neuronal pathways.[155]
Prevents neuroinflammation	Reduces depressive behaviour in rodent models by maintaining higher concentrations of IL-4, which is known to modulate microglial activity.[156] Associated with decreased levels of TNF-α and IL-1β in the hippocampus of LPS-induced rats.[155]
Reduces intestinal inflammation and improves permeability	Modification of gut microbiota by probiotics ameliorated systemic responses in an LPS-induced animal model.[155] Probiotics also prevented stress-induced degradation in colonic mucosa and restored tight junction barrier integrity.[151]
Attenuates HPA-axis response to stress	Decreases corticosterone, adrenaline and noradrenaline levels in chronically stressed mice, suggesting probiotics affect neuronal circuits implicated in the HPA-axis response.[151] Probiotics also prevent the perception of visceral pain associated with chronic stress.[153]
Neurotransmitter modulation	Reduces the activity of enzymes responsible for the conversion of tryptophan to kynurenine, leading to higher levels of serotonin.[152] These effects may be facilitated by indirect immune or endocrine-mediated mechanisms or by directly modulating tryptophan metabolism in the GIT.

exert positive effects on the vagus nerve.[149] The probiotic formulation Cerebiome® has been established in the literature to attenuate stress and reduce symptoms of depression. While not explicitly understood, several mechanisms have been proposed to explain the beneficial effects observed in psychopathological disorders.

Cerebiome® is a unique probiotic containing *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175, which has been clinically trialled for stress and mood support.

Cerebiome® has been shown to reduce serum levels of pro-inflammatory cytokines, including interferon-γ and TNF-α, and demonstrated a significant increase in anti-inflammatory cytokines, such as IL-4 and IL-10.[146,150] Cerebiome® also mitigated the effects of chronic stress in mice by preventing the increase in corticosterone, adrenaline, and noradrenaline and by increasing levels of BDNF in the hypothalamus.[151] It has also been shown to reduce the activity of enzymes responsible for converting tryptophan to kynurenine, leading to higher levels of serotonin and thus exerting their antidepressant effects.[152] The impact on the stress response by these two probiotics may be due to the regulation of glucocorticoid negative feedback, thus attenuating the hyperresponsiveness of the HPA-axis.[153] The probiotic combination also prevented the perception of visceral pain associated with chronic stress more significantly than either probiotic alone, suggesting each strain has specific actions at the peripheral and central level.[153]

Clinical indications

Depression

There is a plethora of evidence indicating that pathophysiological pathways involved in depression are associated with increased intestinal permeability and inflammatory activation.[154] It is well documented that pro-inflammatory cytokines can induce depression and elicit behavioural changes, such as decreased locomotion, reduced food intake, diminished social interactions, and impaired learning and memory.[157] Inflammatory cytokines, such as TNF-α and IL-1β have been associated with neuroendocrine changes and may, in part, be responsible for the stress response at the HPA-axis.[158] Furthermore, exposure to pro-inflammatory cytokines decreases epithelial barrier function and may contribute to chronic inflammation associated with depression. Given the proposed mechanisms of Cerebiome®, various clinical trials have demonstrated beneficial effects in patients with low-to-moderate depression and MDD.[152,159-161]

In a randomised, double-blind, placebo-controlled study, the probiotic supplement was used alongside conventional antidepressant therapy in 110 patients with mild-to-moderate depression.[152] Compared to placebo, the treatment group showed a significant decrease in the Beck Inventory Depression Scores ($p = 0.042$) and blood kynurenine/tryptophan ratios. A follow-up analysis revealed that the probiotic supplementation improved appetite in depressed patients ($p = 0.025$), suggesting these probiotic species may exert their antidepressant effects through serotonin modulation ($p = 0048$).[161] An additional double-blind RCT found the probiotic formula to significantly increase serum BDNF levels compared to a prebiotic ($p < 0.001$) and placebo ($p = 0.021$) in 78 patients with low-to-moderate depression.[160] These results inversely correlated with depression severity compared to placebo ($p < 0.001$), suggesting *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 may improve MDD by influencing the gut-brain axis and altering the expression of BDNF. An open-label study also observed positive effects on mood, anxiety, anhedonia, sleep quality and perceived stress after probiotic supplementation in 10 treatment-naïve patients with MDD.[159] These implications have particular importance for populations for whom antidepressant medications may be contraindicated, such as children, pregnant and breastfeeding women, and people cautious of drug interactions.

Clinical Indications

Cerebiome®


Depression


Anxiety


Stress


Neurodegeneration

Stress and anxiety

Chronic stress is a common denominator in several gastrointestinal disorders and is a key player in gut-brain axis dysregulation.[162] Several studies have indicated that stressful life events affect different gastrointestinal functions such as reflux, gastric emptying, motility, colonic transit, mucosal function, and visceral pain perception. The association between microbiota and the stress response has been demonstrated in animal studies where germ-free mice showed increased HPA-axis responsiveness to stress compared to specific pathogen-free mice.[163] Further studies suggest the gut microbial colonisation process induces neurochemical changes in the brain and initiates signalling pathways that affect anxiety-like behaviour.[164] Outcomes of preclinical and clinical trials suggest psychological and neurological stress impacts the microbiota and reduces particular probiotic species, namely *Lactobacillus* and *Bifidobacteria*.[165]

Two clinical trials have demonstrated the beneficial effects of Cerebiome® in gastrointestinal and psychological stress-related disorders. In the first human clinical study involving healthy stressed adults, the probiotic combination statistically reduced stress-induced gastrointestinal symptoms such as abdominal pain ($p = 0.004$) and nausea ($p = 0.009$) compared to placebo.[166] Moreover, flatulence and bloating tended to diminish in subjects receiving the probiotic supplement ($p = 0.09$). In a double-blind, placebo-controlled, randomised parallel study, the probiotic formula administered for 30 days significantly alleviated psychological distress as measured by the Hopkins Symptom Checklist ($p < 0.05$) and the Hospital Anxiety and Depression Scale ($p < 0.05$) in 66 individuals.[165] Specifically, significant improvements were perceived in somatisation, depression, anger-hostility, anxiety and problem-solving. Beneficial properties of the probiotic formulation were also correlated with decreased levels of urinary free cortisol ($p < 0.05$), suggesting attenuation of HPA-axis response to daily stressors.[165]

These findings highlight the important role of bacteria in the bi-directional communication of the gut-brain axis and suggest probiotic treatment with Cerebiome® may prove to be a practical therapeutic alternative in stress-related disorders.

Other potential indications

Neurodegenerative diseases

Apoptosis and neuroinflammation are regarded as significant factors in the pathogenesis of neurodegenerative disorders.[167] Specific proteins, such as the B cell lymphoma (Bcl-2) family and caspases, and the upregulation of various inflammatory mediators, including TNF-α, are involved in the apoptotic process and contribute to amyloidogenesis in the brain.[168,169] Given that alterations in the gastrointestinal microbiota play a critical role in developing neurological diseases, manipulating intestinal flora through probiotics is a feasible strategy against neurodegenerative diseases.[170]

The reduction of apoptosis in several brain areas and changes in memory have been observed in animal models of depression after probiotic consumption.[171] Prophylactic treatment with Cerebiome® alleviated LPS-induced apoptosis by increasing Bcl-2 protein expression (anti-apoptotic) and attenuating caspase-3 activation in the hippocampus.[172] A previous study by the same authors showed the probiotic formulation decreased serum and hippocampal levels of IL-1β and TNF-α while also attenuating the detrimental effects of LPS on memory.[172] Although the exact underlying molecular mechanism remains unknown, the beneficial effect of these probiotic species may be related to its anti-inflammatory properties. Human clinical trials are essential to confirm these results; nonetheless, Cerebiome® may play a beneficial role in managing neurodegenerative conditions and should be considered an adjunct treatment option.

Dosage safety and clinical tips

Dosing – The daily dose of saffron in most clinical trials was 30mg per day. Up to 500mg has also been clinically trialled for various conditions. In the literature, daily doses of up to 1.5g of saffron are considered safe.[24] There have been reports that doses of >10g per day are toxic and have been used as an abortifacient.

The dosage of Cerebiome® in clinical trials ranged from 3 billion CFU (2.7 billion of *Lactobacillus helveticus* R0052 and 300 million of *Bifidobacterium longum* R0175) up to 10 billion CFU per day.

Contraindications and adverse effects – There are no known cautionary drug interactions for saffron. Further, the combination of antidepressants (fluoxetine, sertraline, citalopram) with saffron may improve depressive symptoms and appears to be safe and effective. Saffron alongside topical ocular hypotensive agents may further decrease intraocular pressure without adverse effects.

There are no known drug interactions with *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175. Across the clinical studies, no side effects have been associated with the probiotic formulation.

Full reference list available at biomedica.com.au

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