

Study Protocol
Version 11 – 06th April 2020

**The efficacy of adjunctive *Garcinia mangostana* linn (mangosteen)
pericarp for bipolar depression: A 24-week double-blind,
randomised, placebo controlled trial.**

Sponsor:

Barwon Health – Deakin University

BH HREC: 15/124

TMC REC: 262

Deakin HREC: 2015-087

Clinical Study Protocol Signature Page

PROTOCOL ID: MANGO BD
VERSION 11 - 06TH APRIL 2020

COORDINATING INVESTIGATOR:
Ms LUCY SAUNDERS
SCHOOL OF MEDICINE, DEAKIN UNIVERSITY

I agree to:

- Implement and conduct this study diligently and in strict compliance with the protocol, ICH Good Clinical Practice Guidelines, the Declaration of Helsinki and all applicable laws and regulations.
- Maintain all information supplied by the Sponsor (or its delegate) in confidence and when this information is submitted to an Institutional Review Board (IRB), Independent Ethics Committee (IEC) or another group, it will be submitted with a designation that the material is confidential.

Coordinating Investigator Printed Name

Signature

Date

Sponsor Representative Printed Name and Title

Signature

Date

Note Confidential information contained herein is made available to you in your capacity as Investigator. It is provided only for review by you, your staff, Institutional Review Board and/or Institutional Ethics Committee members, or regulatory authorities. Except as necessary to obtain properly informed consent for participation, it is expected that there will be no disclosure to other persons.

Table of Contents

1.	Preamble	6
2.	Introduction	6
2.1.	Overview	6
2.2.	Bipolar Depression	7
2.3.	Inflammation, oxidative stress and neurogenesis	7
2.4.	Mangosteen (<i>Garcinia Mangostana</i> Linn)	8
2.5.	Pilot Data	9
3.	Study Objectives/Hypothesis	10
3.1.	Aims/Hypothesis	10
3.1.1.	Aims	10
3.1.2.	Primary Hypothesis	10
3.1.3.	Secondary objectives/hypotheses	10
4.	Variables and Instruments	11
4.1.	Study Procedure	11
4.2.	Exposure measures – <i>Garcinia mangostana</i> Linn & Placebo	11
4.3.	Enrolment Demographic Variables	11
4.4.	Efficacy Variables	12
4.5.	Assessment of inclusion and exclusion criteria	13
4.5.1.	Inclusion Criteria	13
4.5.2.	Exclusion Criteria	13
4.6.	Withdrawal Criteria	14
4.7.	Assessment of safety	14
4.8.	Assessment of predictors and moderators	14
4.9.	Exploration of biological correlates	14
	Blood specimen	16
5.	Study Design and Plan	17
5.1.	Setting	17
5.2.	Sample	17
5.3.	Study Design	17
6.	Procedure	17
6.1.	Identifying potential participants	17
6.2.	Obtaining informed consent	18

6.3.	Revocation of informed consent	18
6.4.	Screening assessment	18
6.5.	Biological correlates	19
6.6.	Randomisation	19
6.7.	Frequency of visits and follow up period	19
6.8.	Withdrawal of participants from treatment or assessment	19
6.9.	Treatments	20
6.10.	Doses and treatment regimens	20
6.10.1.	Labelling	20
6.10.2.	Storage	20
6.10.3.	Accountability	20
6.11.	Blinding and unblinding the study	20
6.11.1.	Methods for ensuring blinding	20
6.11.2.	Methods for unblinding the study	20
6.12.	Prohibited concomitant treatment(s)	21
6.12.1.	Prohibited medication prior to the study:	21
6.12.2.	Prohibited medication during the study:	21
6.13.	Safety measures and variables	21
6.13.1.	Adverse event	211
6.13.2.	Serious adverse event	21
6.13.3.	Reporting of serious adverse events	22
6.14.	Other safety measurements and variables	22
6.15.	Managing suicidality	23
7.	Procedures in case of emergency or pregnancy	23
7.1.	Procedures in case of medical emergency	23
	The chief investigator (or their delegate) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. A medical emergency usually constitutes an SAE and should be reported.	23
7.2.	Procedures in case of pregnancy	23
8.	Data Management	23
8.1.	Documentation	23
8.2.	Archiving	23
9.	Statistical Methods and determination of sample size	24
9.1.	Description of analysis sample	24

9.2.	Method of statistical analysis	24
9.3.	Sample size and power	24
10.	Ethics	25
10.1.	Ethics review	25
10.2.	Documentation	25
11.	Publication Policy	25
12.	Investigators	26
12.1.	Chief Investigator:	26
12.2.	Coordinating Investigator:	26
12.3.	Principal Investigators:	26
13.	References	28

1. Preamble

The protocol outlined below is for “The efficacy of adjunctive *Garcinia mangostana* linn (mangosteen) pericarp for bipolar depression: A 24-Week double-blind, randomised, placebo controlled trial.” This trial is to be run at The Melbourne Clinic and Albert Road Clinic in Melbourne, Barwon Health in Geelong and Toowong Specialist Clinic in Brisbane. Data collected from this trial will be collated for analysis and reporting purposes.

2. Introduction

2.1. Overview

Bipolar disorder is a highly debilitating illness often characterized by suboptimal recovery with conventional treatment. While the disorder has two illness phases; mania and depression, it is the depressive phase that dominates the patient’s life, confers the greatest burden of disease and is associated with the highest risk of suicide. The depressive phase of the disorder is also more refractory to current therapies, which are generally more efficacious in mania. Thus, multiple agents are often required to achieve an adequate level of recovery.

Bipolar disorder is further characterised by perturbed oxidative biology which include reduction in antioxidants levels (glutathione and glutathione transferase) and a concomitant rise in oxidative stress markers (e.g. malondialdehyde). ***Critically, mood stabilising treatments can restore redox balance, and redox active agents such as N-acetylcysteine have antidepressant actions in bipolar disorder.*** Similarly, bipolar disorder manifests systemic inflammation and changes in neuroprogressive markers such as Bcl-2, an apoptotic protein, and mitogen-activated protein kinase (MAPK). This current understanding of the pathophysiology of bipolar disorder supports the search for novel therapeutics affecting the pathways of inflammation, oxidative biology, apoptosis and neurogenesis. Buttressed by robust pilot data ***we propose to use a readily available, tolerable, natural adjunctive therapeutic for bipolar disorder that targets all the aforementioned pathways.***

Garcinia mangostana* Linn**, commonly known as mangosteen, is a fruit that grows mainly in South East Asia. The fruit is contained within a husk known as pericarp, containing a multitude of bioactive agents including xanthenes, polyphenols, tannins and epicatechins. These components reduce oxidative stress and inflammation and improve neurogenesis, making mangosteen a highly suitable agent to explore as an adjunctive therapy for bipolar depression. Philosophically similar to the proposed study, we initially showed that N-acetylcysteine (also targeting oxidative, inflammatory and neurogenesis pathways) reduced the symptoms of schizophrenia including depression. Subsequently, we capitalised on this, demonstrating N-acetylcysteine’s efficacy in bipolar depression, offering further support for the targeting of these pathway. ***Critically, we have pilot data from a double blind randomised placebo-controlled pilot in 76 participants with schizophrenia, indicating that 180 days of mangosteen (1000 mg/day) is beneficial for the treatment of depressive symptoms, with an effect size of 1.19

2.2. Bipolar Depression

Bipolar disorder (BD) has two illness phases; mania and depression. Individuals with bipolar disorder spend three times longer in the depressive phase of the disorder than the manic phase (1). It is the most lethal phase of the disorder, as it confers substantial suicide risk (2). It is highly disabling, and causes marked occupational and social impairment and reduced quality of life (3). Complicating this, it is the phase of illness where the efficacy of established treatments is weakest.

The three largest modern and most methodologically rigorous trials of antidepressants in bipolar disorder (paroxetine, paroxetine or bupropion and agomelatine) have all been negative (4-6). Antidepressants also confer a documented risk of induction of mania. There is little quality evidence for psychological therapy of acute bipolar depression (7). Lithium, a mood stabiliser, is less useful in depression than in mania, and the evidence for the mood stabiliser valproate in depression is equivocal (8). Some, but not all, atypical antipsychotics demonstrate efficacy in bipolar depression. All of these agents have substantial tolerability issues (9). We have shown that even electroconvulsive therapy (ECT), considered a 'last resort' option for severe or refractory depression, is less effective in bipolar than unipolar (major) depression (10). Thus, the unmet clinical need is greatest in bipolar depression.

2.3. Inflammation, oxidative stress and neurogenesis

Multiple studies have linked BD's pathophysiology with inflammation, and immune dysregulation appears to be a common pathway whereby many of the environmental factors that lead to risk for mood disorders are transduced (11). Immune alterations are associated with symptom severity, acute mood episodes, illness progression, metabolic disturbances, drug effects, increased prevalence of autoimmune and allergic disorders, as well as with neurotrophic alterations in patients (12). Acute episodes are characterized as pro-inflammatory states based on findings of increased peripheral levels of pro-inflammatory cytokines on depression, such as interleukin (IL)-6 and tumor necrosis factor alpha (TNF- α), and of IL-2, IL-4, IL-6, and TNF- α in mania (13, 14). Moreover, meta-analyses have reported an increase in the levels of TNF- α , soluble TNF receptor type 1, soluble IL-2 receptor, and IL-1 receptor (15). BD can thus be characterized as a systemic inflammatory disease, even though the exact mechanisms by which these alterations take place are not yet known.

The primary antioxidants in the brain, glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) have all been reported to be altered in bipolar disorder (16). In consort, there are consistent findings of increased markers of oxidative stress, the most robust being peroxidation of cellular lipids. Oxidative damage to both proteins (protein carbonylation) and DNA, are also consistently shown (17).

Regarding neurotrophins, serum brain derived neurotrophic factor (BDNF) levels are reduced in individuals with BD during manic and depressive episodes, and its levels have been negatively correlated with the severity of symptoms (18). In addition, symptom remission has been associated with normalization of serum BDNF levels (19), suggesting that this protein may be a marker of illness activity in BD. Lastly, markers of apoptosis such as S100B and caspase-3 activity are increased in bipolar disorder (20).

With research implicating a variety of factors in addition to monoamine abnormalities, such as oxidative stress and markers of neuroprogression (apoptosis and reduced neurogenesis) new therapeutic targets can be identified and ultimately better treatment options provided to people with bipolar depression (21). Our previous trials using adjunctive therapies (N-acetylcysteine) have successfully shown that targeting oxidative stress, inflammation and neurogenesis, in addition to monoamines, is efficacious in treating bipolar depression, providing a mechanistic proof-of-principle of this approach. The translational and implementation framework of the current study is based on the successful model of using N-acetylcysteine as an adjunctive therapy. In this model, we first found that N-acetylcysteine was efficacious in schizophrenia, including in depressive symptoms in that disorder, opening the door to us further proving that it was efficacious in treating depression in bipolar disorder (22).

2.4. Mangosteen (*Garcinia Mangostana* Linn)

Garcinia mangostana Linn, more commonly referred to as mangosteen, has been used for centuries as a traditional medicine in South East Asia. In recent years mangosteen pericarp and its bioactive compounds have been shown to exert anti-oxidative, anti-inflammatory and neuro-protective effects which are germane to bipolar disorder (23). Pre-clinical studies both *in vitro* and with animals support the therapeutic potential of mangosteen pericarp in bipolar depression. In conjunction with our recent pilot study, trialling mangosteen pericarp in bipolar depression is warranted.

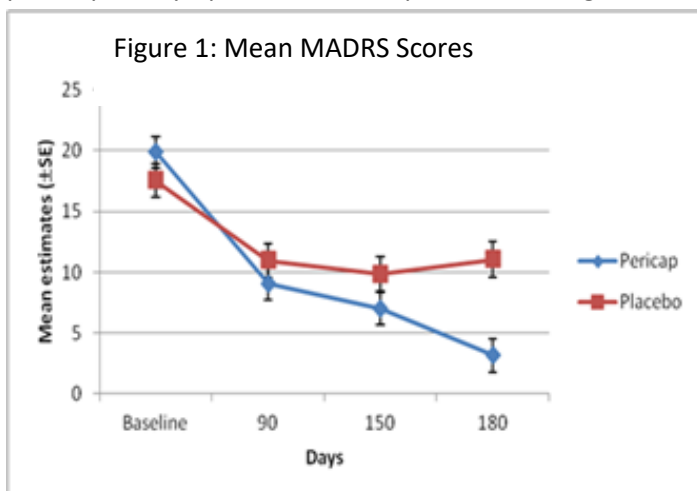
Mangosteen pericarp reduces pro-inflammatory cytokines such as IL-6 and TNF- α , to suppress cyclooxygenase and nitric oxide synthase and to attenuate MAPK levels (24). These anti-inflammatory effects of mangosteen pericarp are thought to occur by inhibition of NF κ B (25). As outlined, this is congruent with pathway changes in bipolar disorder and the efficacy of anti-inflammatory/oxidant treatment for bipolar depression has been demonstrated in our previous studies with N-acetylcysteine. The anti-oxidative effects of mangosteen pericarp and its bioactive ingredients, mostly polyphenolic derivatives, are also well known. Mangosteen pericarp inhibits lipid peroxidation and glutathione (GSH) depletion, leading to reduced levels of oxidative markers (e.g. malondialdehyde) (26). Mangosteen pericarp provides neuro-protection by scavenging peroxides and peroxynitrite, preventing excito-toxicity-induced oxidative stress, and inhibiting disruption of mitochondrial function (27). Mangosteen pericarp can also affect neuroprotection via modulation of apoptosis. Alterations in neurogenesis in bipolar disorder have been described (21) and therapeutic agents (i.e. mood stabilisers) have been shown to affect these pathways (28).

α -Mangostin was shown to up-regulate mitogen activated protein kinase (MAPK), extracellular signal-regulated kinases (ERK), Akt, c-Myc/Max and p53 cell signalling pathways. Downstream these pathways affect cell growth, cell proliferation, cell motility, survival, protein synthesis, and transcription via Bcl-2 and p53, two proteins that regulate cell death pathways (29). Lastly, mangosteen pericarp was shown to affect the levels of neurotransmitters and their receptors including acetylcholine, acetylcholine esterase and 5-hydroxytryptamine 2A receptors (30, 31).

The study will use the pericarp of mangosteen, the dark layer between the rind and the flesh (Figure 1). The pericarp contains over 85 bioactive constituents including α - and β -mangostin, with the former being present at the highest concentrations (29, 31). The xanthone constituents, predominantly α -mangostin, are also the most extensively characterised. Xanthones in mangosteen pericarp possess polyphenol derivatives which act as antioxidants (29). Changes in antioxidant levels are well-characterised in bipolar disorder and the efficacy of antioxidant treatment for bipolar depression has been demonstrated in other studies (e.g. N-acetylcysteine). Critically, α -mangostin affects glutathione levels and glutathione peroxidase activity. By targeting antioxidant pathways, mangosteen pericarp may improve symptoms. Interestingly, some xanthones have anticonvulsant properties and it's noteworthy that many mood stabiliser treatments are repurposed anticonvulsants (32).

2.5. Pilot Data

In addition to the preclinical literature indicating mangosteen pericarp has neurobiological activity that could be useful to treat bipolar depression, we also have pilot data to suggest that adjunctive mangosteen pericarp treatment affects mood symptoms. We have just completed a double blind, randomised placebo controlled trial of mangosteen for individuals with schizophrenia (accepted for publication in Molecular Psychiatry). Seventy-six participants took part in the 24-Week treatment trial where 1000 mg/day of mangosteen pericarp (or placebo) was provided to participants who were interviewed over the course of the study to determine changes in symptoms. The study was designed to explore the effects of mangosteen pericarp on symptoms of schizophrenia and significant reductions in positive and negative symptoms



(based on the Positive and Negative Symptoms Scale) were seen. The study also included a validated mood rating scale; the Montgomery Asberg Depression Rating Scale (MADRS). The improvements in depressive symptoms (Figure 1) showed a highly significant clinical effect (Cohen's $d=1.19$). Indeed, these effects are larger than most trials investigating antidepressant treatment for bipolar depression. Furthermore, there were no reported differences in the

occurrence of adverse events, indicating mangosteen pericarp was safe and well-tolerated.

The pathway from schizophrenia to bipolar disorder in drug discovery is well worn. Many agents such as atypical antipsychotics were discovered this way by our team. Atypical antipsychotics such as olanzapine and risperidone were initially used in schizophrenia and based on our first-in-kind trials, prior to any industry interest, are now a first line treatment for bipolar disorder (33, 34). We also have a track record in first discovering N-acetylcysteine as a beneficial treatment for mood, initially finding that it reduces depression in schizophrenia, then proving efficacy in bipolar disorder. Indeed, overlaps in the neurobiology between bipolar disorder and schizophrenia is concordant with efficacy across disorders. As an exemplar and proof of concept, we have shown that N-acetylcysteine, which shares effects on inflammation, oxidative stress, neurogenesis and apoptosis, reduces symptoms of depression in bipolar disorder and depression. The veracity of this approach is demonstrated by our high-impact trials, highlighted in our May 2015 lead article on pathways to drug discovery in *The American Journal of Psychiatry* (35). Taken together, the scientific literature and pilot data suggest mangosteen pericarp may be a useful adjunctive treatment for bipolar disorder.

3. Study Objectives/Hypothesis

3.1. Aims/Hypothesis

3.1.1. Aims

The primary aim of this study is to investigate the efficacy of adjunctive mangosteen pericarp 1000mg/day for the treatment of bipolar depression using a 24-Week randomised, placebo-controlled trial. The primary outcome measure will be the change in severity of mood symptoms, measured using the Montgomery Åsberg Depression Rating Scale (MADRS). Secondary outcomes include global psychopathology, substance use, functioning, quality of life, and safety and tolerability data. A follow-up interview will be conducted 4 weeks post-treatment to determine any outcomes following cessation of the trial agent.

3.1.2. Primary Hypothesis

- i) 24-Weeks of adjunctive mangosteen pericarp treatment will be superior to placebo in depression, as measured using the MADRS.

3.1.3. Secondary objectives/hypotheses

- ii) 24-Weeks of adjunctive mangosteen pericarp treatment will be superior to placebo for reducing symptomology (Clinical Global Improvement for Bipolar Disorder; CGI-BP, Hamilton Anxiety Rating Scale; HAM-A, Bipolar Depression Rating Scale; BDRS, Young Mania Rating Scale; YMRS, Patient Global Impression Scale; PGI), Quality of Life (Quality of life Enjoyment and Satisfaction Questionnaire – Short Form; Q-LES-Q-SF) and functioning (Social and Occupational Functioning Scale; SOFAS, Range of Impaired Functioning Tool; LIFE-RIFT) and cognition (digit span forwards and backwards, trail making and symbol digit test)

- iii)* Those taking the mangosteen pericarp treatment will have better outcomes 4 weeks post-treatment discontinuation, based on the same symptomology, quality of life and functioning scales (as during the treatment phase) than those taking the placebo
- iv)* Those taking mangosteen pericarp treatment will have reduced oxidative stress (malondialdehyde) and inflammatory (IL-6, TNF- α and CRP) markers and improved markers of neuroprotection (Bcl-2), in peripheral (blood) samples compared to placebo,
- v)* Clinical changes will correlate with discernible changes in oxidative stress markers (malondialdehyde) inflammatory markers (IL-6, TNF- α and CRP) and improved markers of neuroprotection (Bcl-2), in peripheral (blood) samples compared to placebo.
- vi)* From both health sector and societal perspectives 24-Weeks of adjunctive mangosteen pericarp treatment will be cost effective compared to placebo where \$50,000 per quality adjusted life year (QALY) is the accepted benchmark for cost-effectiveness in Australia.
- vii)* Those taking the mangosteen pericarp treatment will have improved cognition as during the treatment phase) than those taking the placebo

4. Variables and Instruments

4.1. Study Procedure

Trial clinicians will contact all participants referred to the trial to schedule an initial face-to-face screening interview. During the initial interview, the trial clinicians establish written consent, that inclusion criteria are satisfied, and confirm the diagnosis using the (SCID-5-RV). All randomised participants will receive two 500mg mangosteen pericarp capsules once a day to a total dose of 1000mg daily (or placebo) with food, in addition to treatment as usual. The mangosteen pericarp and placebo will be encapsulated and matched in appearance. Dose changes to existing medications (either increases or decreases in dose) or switch in medication will be accepted, documented and participants will be allowed to continue on the trial.

4.2. Exposure measures – *Garcinia mangostana* Linn & Placebo

From visit one, participants will be instructed to return unused medication. The amount of medication dispensed and returned will be calculated and documented. This data will be used to calculate compliance with medication for analysis purposes.

4.3. Enrolment Demographic Variables

The following variables will be assessed for enrolment in the study and for demographic purposes:

- Demographics (age, gender, marital status, educational attainment)
- Onset of prodromal symptoms

- Duration of current episode and illness
- Age at first episode and number of previous episodes
- Frequency of hospital admissions and any precipitating factors
- Presence of comorbid disorders and other axis I and II disorders (DSM-5)
- Medical history
- Current and previous medication
- Family psychiatric history
- Smoking, alcohol and illicit substance behaviours
- Use of dietary supplements

4.4.Efficacy Variables

The primary outcome variable will be the MADRS total score at the end of the 24-week treatment phase.

Secondary outcomes include the Bipolar Depression Rating Scale (BDRS), Young Mania Rating Scale (YMRS) and Hamilton Anxiety Rating Scale (HAM-A) scores. Participant life satisfaction will be rated on the self-reporting scale (Q-LES-Q). Functioning measures will include the Social and Occupational Functioning Assessment Scale (SOFAS), and Longitudinal Interval Follow-Up Evaluation-Range of Impaired Functioning Tool (LIFE-RIFT). The Clinical Global Impressions Bipolar Version (CGI-BP) will be used to rate clinician judgement for improvement and severity of illness. The Patient Global Impression Scale (PGI) will be used to rate participant perception. We will ask participants to complete a number of pen and paper based cognitive tasks; digit span forwards and backwards, trail making and the Symbol Digit task.

In order to undertake a cost-utility analysis (CUA), gains in utility for the participants will be measured in the study. The most common metric developed to measure utility gains from health interventions is the QALY. A QALY is simply calculated by multiplying the period of time spent in a particular health state by the “utility” or preference value assigned to that health state measured on a scale from 0 (denoting death) to 1 (denoting full health). There are different techniques of deriving utility however the simplest is via the use of a multi-attribute utility instrument which is basically a health related quality of life measure (HRQoL) which has pre-determined “weights” assigned to the health states included within the measure – this is called indirect elicitation of utility. We propose to use the AQoL4D to assess any utility based HRQoL gains. It comprises four separately scored dimensions, consisting of independent living, relationships, mental health, and senses. It consists of 12 items and takes 2 minutes to complete. The main utility algorithm of the AQoL has been scored using the time trade off method and the questionnaire has sound psychometric properties. The AQoL4D will be administered to study participants at baseline, 12 and 24 weeks. Scoring will be completed with the algorithm provided by the developers of the instrument obtained from: <http://www.aqol.com.au/scoring-algorithms.html> .

To measure the healthcare services used by participants during the course of the trial, but outside of the study assessments, a resource use questionnaire (RUQ) will be employed. The RUQ will ask participants to provide information on hospitalisations, consultations with health care providers, and other related health

services utilised. The RUQ also asks questions regarding lost paid and unpaid productivity. The data collected will be combined with other data derived from the trial (i.e. medications utilised) and then valued using standard Australian unit costs from the Medicare and Pharmaceutical Benefits Schedules, the Independent Hospital Pricing Authority, and wage rates from The Australian Bureau of Statistics.

4.5. Assessment of inclusion and exclusion criteria

4.5.1. Inclusion Criteria

- Aged 18 years or older,
- Have a DSM-V diagnosis of bipolar disorder I or II, or bipolar disorder not elsewhere classified (NEC),
- Currently be in a major depressive episode on SCID-5-RV
- Score at ≥ 20 on the MADRS
- Participants currently under therapy for bipolar disorder will need to have been on that primary therapy for at least four weeks prior to randomization,
- Have the capacity to consent to the study and comply with study procedures,
- Be using effective contraception if female, sexually active and of childbearing age,
- Be able to speak, read, write and understand the English language,
- Participants will be required to nominate a current treating physician and will not be eligible to enter the study until one is identified.
- If a researcher is in doubt at any time about an inclusion criterion point please consult an investigator.
- If there is a delay of >7 days between screening and baseline assessments, or baseline assessment and medication commencement, the inclusion scale (MADRS) should be administered again to ensure the participant still meets eligibility criteria.

4.5.2. Exclusion Criteria

- Participants with a known or suspected active systemic medical disorder,
- Individuals who are pregnant or lactating (participants will be requested to conduct a urine pregnancy test if sexually active and of child-bearing age),
- Participants currently enrolled in any other intervention study will be excluded,
- Individuals who are intolerant, allergic to or have had an anaphylactic reaction to any components of the preparation,
- Inability to comply with either the requirements of informed consent or the treatment protocol.

4.6. Withdrawal Criteria

Withdrawal from the trial will occur if the participants cease taking their trial medication for seven consecutive days or if the participants cease effective contraception or becomes pregnant or if the participant commences new Electroconvulsive Therapy (ECT) treatment. Dose changes to existing medications (either increases or decreases in dose), or addition or removal of an agent will be accepted and participants will be allowed to continue with the trial. Participants will be withdrawn from the study if they withdraw consent or at the discretion of the researcher given adverse events or loss to follow-up. Serious adverse events will not require automatic withdrawal from the study.

4.7. Assessment of safety

All adverse events will be recorded, intervened according to medical assessment, and monitored. Participants are asked open-ended questions about how they have been feeling in general and if they have any concerns regarding their health or the study medication. The DSMB will review the study reports and determine any safety issues if they arise.

4.8. Assessment of predictors and moderators

- The Standardised Assessment of Personality – Abbreviated Scale (SAPAS) will be used to identify those with suspected personality disorders and this will be used as a covariate in the analysis and also used as a variable of interest in examining treatment response.
- The Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2) is a self-administered paper based modification of the food frequencies questionnaire developed by the Cancer Council to measure dietary intake. Diet quality can then be used as a covariate in the analysis and also used as a variable of interest in examining treatment response.
- Analysis of biomarkers of oxidative and inflammatory markers will be used to determine how treatment response (if present) correlates to changes in biological markers.
- Changes in biomarkers will be used to explore the underlying pathophysiology of depression.

4.9. Exploration of biological correlates

Blood samples will be obtained at baseline and week 24, to be stored and later analysed for biological correlates based on preclinical evidence including markers of antioxidant defence (including antioxidant levels), oxidative stress (including markers of lipid peroxidation and protein carbonylation), and markers of inflammation (including IL1, IL6, CRP and TNF α), which interact with oxidative stress.

Table 1: Instruments / procedures to be implemented at each study visit:

VISIT	1 Baseline/ Screening *	2 Interim assessment	3 Interim assessment	4 Interim assessment	5 Interim assessment	6 Interim assessment	7 Primary End- point	8 Post- discontinuation follow-up
WEEK	0	4	8	12	16	20	24	28
SCREENING AND CONSENT								
Informed consent	X							
Inclusion/exclusion criteria	X							
Demographics	X							
Medical history	X							
Psychiatric and family history	X							
Concomitant medication	X	X	X	X	X	X	X	X
Pregnancy test (females only)**	X							
SCID-5-RV	X							
SAFETY								
Adverse Events	X	X	X	X	X	X	X	X
EFFICIACY								
MADRS	X	X	X	X	X	X	X	X
BDRS	X	X	X	X	X	X	X	X
YMRS	X	X	X	X	X	X	X	X
HAM-A	X	X	X	X	X	X	X	X
CGI-S	X	X	X	X	X	X	X	X
CGI-I		X	X	X	X	X	X	X
PGI		X	X	X	X	X	X	X
COGNITION								
Digit span forwards	X						X	
Digit span backwards	X						X	
Trail making	X						X	
Symbol digit task	X						X	
FUNCTIONING AND QUALITY OF LIFE								
Q-LES-Q	X	X	X	X	X	X	X	X
SOFAS	X	X	X	X	X	X	X	X
LIFE-RIFT	X	X	X	X	X	X	X	X
ASSESSMENT OF PREDICTORS AND MODERATORS								
SAPAS	X							
DQES V.2	X							
HEALTH ECONOMICS								

VISIT	1 Baseline/ Screening *	2 Interim assessment	3 Interim assessment	4 Interim assessment	5 Interim assessment	6 Interim assessment	7 Primary End- point	8 Post- discontinuation follow-up
AQOL-4D	X			X			X	
HEALTH UTILISATION QUESTIONNAIRE	X			X			X	
EXPLORATION OF BIOLOGICAL CORRELATES								
Blood specimen	X						X	
OTHER								
Drug Dispensation	X	X	X	X	X	X		

* Baseline and screening interviews may be split to accommodate participant time.

**If participant is not sexually active or the treating clinician feels it is not warranted, this is not compulsory.

5. Study Design and Plan

5.1. Setting

The study will be conducted at The Melbourne Clinic and Albert Road Clinic in Melbourne, Barwon Health in Geelong and Toowong Clinic in Brisbane. Sponsorship for the study will be provided by Barwon Health. The teams at all recruiting sites have strong links with established clinical trial teams with track records in innovation including partners on the pilot trial.

5.2. Sample

Participants will be sought from private and public healthcare settings as well as community recruitment. Participants (N=150) aged 18 and above years with moderate to severe bipolar depression will be studied. Participants must meet the DSM-5 criteria for bipolar disorder I or II, or bipolar disorder not elsewhere classified (NEC), determined using the Structured Clinical Interview for DSM Disorders (SCID-5-RV), meeting criteria of a Montgomery Asberg Depression Rating Scale score of ≥ 20 and current major depressive episode on the SICD-5. The participants will be assigned randomly and consecutively to treatment with mangosteen pericarp or placebo in a double blind fashion in addition to treatment as usual (TAU). All participants will remain on their usual treatment for the course of the trial.

5.3. Study Design

This is a 24-week, double blind, randomised, placebo-controlled trial of adjunctive *Garcinia mangostana* Linn in the treatment of bipolar depression (n=150). The duration of the current trial will be 24 weeks concordant with our pilot data. A follow-up interview will be scheduled 4 weeks after completion of the study. The trial will be conducted following approval from the relevant ethics committees, and conducted in accordance with the Good Clinical Practice guidelines. The trial will be conducted according to Australian Clinical Trial guidelines and the National Ethical guidelines for Human Research. This protocol was developed in accordance with Standard Protocol Items Recommendations for Intervention Trials (SPIRIT) 2013 guidelines, and findings will be reported using CONSORT guidelines. All participants will give informed consent prior to enrolment. Participants will be withdrawn from the study if they withdraw consent or at the discretion of the investigators.

6. Procedure

6.1. Identifying potential participants

All sites have a clinical trials teams dedicated to the successful conduct and completion of clinical trials in psychiatric disorders. We have established protocols for maximising recruitment and retention rates. We utilise various recruitment avenues including advertisement through local newspapers, flyers in medical waiting rooms, pharmacies and university campuses, and direct contact with potential referral sources. These consist of general practitioners, community mental health teams, the disability support sector, support groups, private psychiatrists and the local psychiatric inpatient unit.

Participants contact the trials team directly via the mangosteen email, by calling local clinicians directly (details on all promotional materials), may be forwarded on through Healthshare – an online health portal, contacted through our centralized database, or may be encouraged by treating mental health professionals to contact the trials team. Participants may also be contacted following referral from their treating physician and will be informed that they are being offered the study through that channel.

6.2.Obtaining informed consent

Participants who are deemed competent to provide informed consent will be included in the study. This competency will be determined by the researcher, in collaboration with the treating physician if required, and will take into consideration a broad range of factors as suggested within the NHMRC *National Statement* on Ethical Research in Humans.

6.3.Revocation of informed consent

All participants have the right to revoke consent from the study at any time and this must be clearly stipulated at the time of consent. Participants may request to have their blood sample destroyed at this time. Revocation of consent forms will need to be completed for all participants (where possible) who withdraw from the study. Revocation of consent will refer to the destruction of clinical data. This is independent of withdrawal of consent, where a participant may choose to withdraw from the study, but have their data used regardless.

6.4.Screening assessment

No study specific procedures or investigations will be performed before the participant has signed and dated the informed consent form. After consent has been obtained, a research assistant will conduct the screening assessment as per Table 1. This will include a full assessment of all inclusion and exclusion criteria. In the event that face-to-face recruitment is not possible due to safety concerns, we will alter recruitment to utilise online methods including Zoom, Skype, or teleconference as a last resort. If screening via an online method, participants will not be asked to provide blood specimens. Participants will be provided with the PICF either in hard copy or electronically, as they prefer. Once received, the participants will have the opportunity to review the information and discuss with anyone they deem relevant. The researcher will review the PICF document over the telephone or via an online medium with the participant and establish consent. Participants either will be able to sign the PICF and return electronically or will be provided with a reply paid envelope to mail hardcopy documents back to the researcher for sighting and archiving. If the participant does not meet one of the requisite inclusion criteria, they will not take further part in the trial. For example, if the initial assessment reveals that a participant does not actually meet a diagnosis of bipolar disorder I or II, or bipolar disorder not elsewhere classified (NEC), or that their MADRS score is lower than 20, or they do not meet current major depressive episode on the SCID-5-RV, then they will be withdrawn based on the fact that they do meet inclusion criteria. These participants will be deemed to be 'screen fails' and will be constituted as such in the CONSORT flowchart.

6.5. Biological correlates

For consenting participants, blood samples will be obtained at baseline and week 24 and will be stored and later analysed for relevant markers based on preclinical evidence including markers of antioxidant defence (including antioxidant levels), oxidative stress (including markers of lipid peroxidation and protein carbonylation), and markers of inflammation (including IL1, IL6, CRP and TNF α) and a range of other sera markers related to general health, which interact with oxidative stress. Participants will have the option to consent to the collection of blood samples to explore RNA expression. We will explore the expression of genes that may be influenced by mangosteen pericarp including metabolic, oxidative, inflammatory and cell cycle genes. For consenting participants, the RNA sample will be collected using PAXGene Blood RNA tubes. In addition to these analyses, Participants will have the option to give unspecified consent for future research. Any future studies will be conducted after approval from a Human Research Ethics Committee. All bloods will be stored in -80°C Freezers for no more than 15 years.

6.6. Randomisation

After the baseline assessment has been completed, the participant will be randomised to either the *Garcinia mangostana* Linn group or the placebo group. Allocation to treatment arm will be randomly assigned in a 1:1 ratio (active to placebo) using permuted block randomisation. An independent researcher utilising a four-to-a-block design will develop the computer-generated randomisation plan. Trial clinicians will allocate packs sequentially, and bottles are identical so as to conceal treatment allocation and blinding. To facilitate the double-blinding process, the trial medications will be dispensed by an independent pharmacist in identical numbers and capsule forms in sealed containers. The statistician and trial clinicians will be blinded to the group allocation.

6.7. Frequency of visits and follow up period

Participants will be assessed at baseline, regular 4-weekly intervals throughout the 24-week trial (weeks 4, 8, 12, 16, 20, 24), and at a post-discontinuation follow-up 4 weeks after completion of the trial as outlined in Table 1. Interviews with participants will take an average of 40-60 minutes. If participants cannot be seen face-to-face due to safety concerns, interviews will be conducted via online platforms such as Zoom, Skype or over teleconference as a last resort. Once enrolled, participants will be reimbursed for travel up to the value of \$20, per visit, or to cover internet expenses. Visits must occur within one week of the pre-determined date (based on baseline visit date). If a visit occurs more than 7 days after the pre-determined date, this will be a deviation from protocol. If participants cannot attend any visit before 2 weeks from the pre-determined date, this visit will be skipped until the next scheduled assessment. A skipped visit will also be a deviation from protocol.

6.8. Withdrawal of participants from treatment or assessment

Participants will be withdrawn from the trial if they; discontinue the trial medication for >7 days; cease effective contraception, become pregnant or withdraw consent.

Discontinuation due to adverse events (both serious and non-serious) may be a criterion for withdrawal, but is not mandatory, and could be either at the request of the participant or the discretion of the investigator. Withdrawal of consent will not require any data to be destroyed. This instead is termed 'revocation of consent' and has an associated form to be completed.

6.9. Treatments

1000 mg/day of *Garcinia mangostana* Linn will be used in the study. Placebo capsules matched in appearance and taste will be used.

6.10. Doses and treatment regimens

Trial medication will be dispensed on a monthly basis, so there will be a total of six dispensations per participant. The medication will be provided to the participant after the baseline visit has been completed and the participant has been randomised. The trial medications will be supplied by the site pharmacy. Participants will be instructed to return all containers to allow capsule counts by the trial monitor and the trial pharmacist. In the event that face-to-face visits are unable to be conducted due to safety concerns, medications will either be delivered to participants front doors or mailed directly to participants. Participants will be provided with reply paid envelopes to post remaining medication back to the researcher.

6.10.1. Labelling

The labelling of study medication will comply with local regulatory GCP requirements.

6.10.2. Storage

All investigational products will be kept in a secure location under appropriate storage conditions. A description of the appropriate storage should be specified on the investigational product label.

6.10.3. Accountability

Concordant with SPIRIT guidelines, adherence will be assessed by pill counts of returned medication packs. Adverse effects are recorded and monitored and will be reviewed by our independent DSMB.

6.11. Blinding and unblinding the study

6.11.1. Methods for ensuring blinding

Blindness will be maintained by ensuring that the packaging, appearance, colour and taste of mangosteen pericarp and placebo capsules are identical. Blindness will also be maintained by the use of an anonymous Participant-ID and not allowing study personnel access to the randomisation code unless in an emergency. The research assistants will remain blinded throughout the entire trial. Analysis of the data will also be conducted blind to the statistician and Investigators.

6.11.2. Methods for unblinding the study

Individual treatment codes, indicating the treatment randomisation for each randomised participant, will be available to the pharmacists at the participating sites. The treatment code must not be broken throughout the study period except in medical emergencies when the appropriate management of the participant necessitates knowledge of the treatment randomisation. All cases of emergency unblinding must be thoroughly documented and require reporting to the DSMB (if that had not occurred previously). Participants will be notified at the completion of all participants in the study, which arm of the study they took part in.

6.12. Prohibited concomitant treatment(s)

6.12.1. Prohibited medication prior to the study:

Any preparation of mangosteen pericarp will be prohibited prior to study entry.

6.12.2. Prohibited medication during the study:

Any preparation of mangosteen pericarp will be prohibited during the trial.

6.13. Safety measures and variables

The definitions of adverse events (AEs) and serious adverse events (SAEs) are given below.

6.13.1. Adverse event

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including washout periods, even if no study treatment has been administered.

6.13.2. Serious adverse event

A serious adverse event is an AE occurring during the treatment phase (i.e. baseline to week 24), of the investigational product, comparator or placebo, which fulfils one or more of the following criteria:

- results in death
- is immediately life-threatening
- requires in-patient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital abnormality or birth defect
- is an important medical event that may jeopardise the patient or may require medical intervention to prevent one of the outcomes listed above.

The causality of SAEs (i.e., their relationship to study treatment) will be assessed by the investigator(s). Note that SAEs that could be associated with any study procedure should also be reported. For such events the casual relationship is implied as "yes".

6.13.2.1. Recording of adverse events

Adverse events will be collected from the time that Informed Consent has been obtained until the end of the 24 week intervention period. At each visit, participants will be asked if they have had any health problems since the previous visit. All AEs will be recorded appropriately, whether or not considered related to the investigational product. This will include AEs spontaneously reported by the participant and/or observed by the staff as well as AEs reported in response to a direct question e.g. "Have you had any health problems since your last visit". The current study also encourages reports of unexpected improvements as part of the AE reporting.

For each AE the following parameters will be described: a description of the event, start and stop date, action taken with regards to investigational product (if applicable), outcome, if the AE caused the patient to discontinue, a statement if the AE fulfils the criteria for a SAE or not, the treating doctor or investigator's assessment of the causal relationship between the event and the investigational product, treatment for the AE and the intensity of the AE.

6.13.2.2. Follow-up of adverse events

After the 24 week intervention period, adverse events will be followed up until either of the following conditions is met:

- The adverse event is resolved; or
- 7 days following the completion of the study or study withdrawal. Participants should be called at this time and told that they should contact their own treating physician if adverse events persist. Further follow-up of an adverse event can occur at the discretion of the treating physician or Investigator.

6.13.3. Reporting of serious adverse events

Reporting of SAEs to regulatory authorities will be done by the investigator (or delegate) in accordance with local regulations. SAEs will also be reported to the relevant HRECs according to policy and guidelines. The project manager and coordinating investigator or their nominated delegates will be responsible for reporting the SAEs to the appropriate authorities. If unblinding is required, a report will also be sent to the Therapeutic Goods Administration, or relevant regulatory body. **Trial staff must report SAE's to the trial coordinator immediately as they are required to be reported to the appropriate regulatory agencies within 24 hours.**

6.14. Other safety measurements and variables

If the participant is female, of child-bearing potential and sexually active, urine pregnancy tests will be conducted at baseline.

6.15. Managing suicidality

All participants will be required to nominate a current treating physician on enrollment into the study and will be notified that that physician may be contacted, should the research team feel it is appropriate. Should a participant express suicidal ideation with a specific plan or intent (a MADRS suicide item score of 5 or 6), the researcher will strongly advise the participant to inform their current treating physician, and will also attempt to contact the treating physician directly. Should the researcher have significant concerns for the immediate safety of the participant (e.g. severe suicidality with immediate risk or a recent, unreported suicide attempt) the researcher will seek advice from the PI, and will take appropriate steps to ensure participant safety (including informing the treating physician and assisting the person to appropriate care, for example by taking the person to the emergency department or calling an ambulance).

7. Procedures in case of emergency or pregnancy

7.1. Procedures in case of medical emergency

The chief investigator (or their delegate) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an SAE and should be reported.**

7.2. Procedures in case of pregnancy

In case of pregnancy, treatment with study drug should immediately be terminated. Pregnancy itself is not regarded as a serious adverse event unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of a contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) will be followed up until week 28 and documented. All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages will also be reported and handled as SAEs. Elective abortions without complications will not be handled as AEs.

8. Data Management

8.1. Documentation

Source data will include both hard copy and electronic questionnaires and measures. The hard copy data will be retained in a locked filing cabinet at each study site. . Electronic data will be directly input into the de-identified eCRF held on REDCap. De-identified data will be entered into a password protected electronic database. Research assistants will be responsible for entering data into the database and will be trained accordingly.

After data cleaning procedures are finished, the clinical trial database will be closed. All data will be exported into the appropriate software to enable statistical analysis.

8.2. Archiving

The coordinating investigator, project manager or their delegate shall arrange for the retention of documentation relating to the trial (source documents, informed consent forms, approvals) for 15 years. Archiving for all sites will take place within the IMPACT SRC at Barwon Health.

9. Statistical Methods and determination of sample size

9.1. Description of analysis sample

The sample to be included in the analyses will be all the participants who give consent to participate in the study who meet all inclusion criteria and no exclusion criteria, and who have taken at least one dose of trial medication and completed at least one post-baseline assessment.

9.2. Method of statistical analysis

All analyses will be conducted in accordance with the International Conference on Harmonization E9 statistical principles, and are based on all randomised participants with at least one post-baseline observation (intention to treat population). Reporting of research findings will be done in accordance to CONSORT guidelines. The statistician responsible for the analysis of outcome data will be blind to group allocation. The primary efficacy analysis will assess average treatment group differences for the primary outcome measure MADRS total score over the entire study period and will use a likelihood based mixed-effects model, repeated measures approach (MMRM). The MMRM model includes the fixed, categorical effects of treatment, investigator, visit, and treatment-by-visit interaction, as well as the continuous, fixed covariates of baseline score and baseline score-by-visit interaction. The MMRM includes all available data at each time point and is the preferred method of analyzing clinical trial data in psychiatry as compared to more traditional repeated measures analysis of variance (ANOVA) and analysis of covariance models (ANCOVA). Planned comparisons will be done with the MMRM models to determine between group differences in change of symptoms measures from baseline to week 24. Results from the analysis of dichotomous data will be presented as proportions, with 95% confidence interval, and Fisher's exact p-value where appropriate. Non-parametric statistics will be used when assumptions for parametric methods are violated. Effect sizes will be calculated using Cohen's guidelines. All tests of treatment effects will be conducted using a two-sided alpha level of 0.05 and 95% confidence intervals. Change scores in symptoms and in biological markers will be derived. Pearson Product Moment Correlations will be used to examine the relationships between the change scores for symptoms and the change scores for biological markers in the two groups. Using Fisher's z transformation, we will test whether the correlations obtained for each group are statistically different in terms of strength.

9.3. Sample size and power

We are aiming for a target sample size of 150 participants. For a two tailed analysis with $\alpha=0.05$, $Z\alpha = 1.96$ and with $\beta=0.2$, $Z\beta = 0.8$, $N=120$, the study should be powered at 80% to detect a true difference in MADRS scale score between the mangosteen pericarp and placebo groups if the effect size is

Cohen's $d=0.362$ or greater. This is a conservative estimate, based on the results of the pilot data. However, in our previous trial of N-acetylcysteine specifically investigating bipolar depression, effect sizes for depressive symptoms (BDRS, MADRS), clinical global impression of depressive severity (CGI BP-depression), as well as all functional measures have shown effects similar to or above the effect size the sample has been calculated for. Based on our previous trials we typically have an attrition rate of 20%. Therefore, although power calculations indicated 120 participants will be sufficient to detect between group differences, we plan to recruit 150 participants to account for attrition.

10. Ethics

10.1. Ethics review

All documentation pertaining to the study must be prepared in accordance with the requirements outlined by the relevant ethics committee. All documentation must then be approved or given a favourable opinion in writing by an HREC as appropriate.

The Coordinating Investigator along with the Trial Coordinator is responsible for informing the IRB or HREC of any amendment to the protocol in accordance with local requirements. In addition, the HREC must approve all advertising used to recruit participants for the study.

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements. The NHMRC National Statement on Human Research will also be utilised.

10.2. Documentation

This study will not commence at any site until the final protocol, informed consent form and other associated documents are approved by the HREC. Any protocol amendments will also be managed in accordance with local ethical requirements. As previously mentioned, the research team will ensure that the participant is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. The participant should be given the opportunity to ask questions and allowed time to consider the information provided. Participants must also be notified that they are free to discontinue from the study at any time.

The principal investigator or their delegate will store the original, signed Informed Consent Form in a secure, locked filing cabinet. A copy of the signed Informed Consent Form will be given to the participant. All researchers are required to sign that their data collection is a true and accurate record.

11. Publication Policy

Final results will be published after termination of the study. The order of authors will be at the discretion of the coordinating investigator. Factors that the coordinating investigator may take into consideration are the following: participation in organising the study, participation in meetings and the on-going development of the study, manuscript production and general involvement in the study.

12. Investigators

12.1. Chief Investigator:

Dr Olivia Dean

IMPACT SRC - Deakin University

P.O. Box 281

Geelong VIC 3220 Australia

Phone: +61 3 4215 3300

Email: oliviad@barwonhealth.org.au

12.2. Coordinating Investigator:

Ms Lucy Saunders

IMPACT SRC - Deakin University

P.O. Box 281

Geelong VIC 3220 Australia

Phone: +61 3 4215 3327

Email: lucy.saunders@deakin.edu.au

12.3. Principal Investigators:

Prof. Michael Berk

Professor of Psychiatry

IMPACT SRC - Deakin University

University Hospital – Barwon Health

P.O. Box 281

Geelong VIC 3220

Phone: +61 3 5226 7450

Email: mikebe@barwonhealth.org.au

Prof. Chee Ng

Professor of Psychiatry

University of Melbourne

The Melbourne Clinic

130 Church St

Richmond VIC 3121

Phone: +61 3 9426 2111

Email: cng@unimelb.edu.au

Prof. Malcolm Hopwood

Professor of Psychiatry

University of Melbourne

Albert Road Clinic

31 Albert Rd

Melbourne, VIC, 3004
Phone: 03 9279 3518
Email: mhopwood@unimelb.edu.au

Dr Jon-Paul Khoo

Psychiatrist
Toowong Specialist Clinic
53 Jephson Street
Brisbane, QLD, 4066
Phone: 07 3720 9700
Email: jon-paul.khoo@toowongclinic.com.au

13. References

1. Judd LL, Akiskal HS, Schettler PJ, Endicott J, Maser J, Solomon DA, et al. The long-term natural history of the weekly symptomatic status of bipolar I disorder. *Arch Gen Psychiatry*. 2002;59(6):530-7.
2. Simpson SG, Jamison KR. The risk of suicide in patients with bipolar disorders. *J Clin Psychiatry*. 1999;60 Suppl 2:53-6; discussion 75-6, 113-6.
3. Dean BB, Gerner D, Gerner RH. A systematic review evaluating health-related quality of life, work impairment, and healthcare costs and utilization in bipolar disorder. *Curr Med Res Opin*. 2004;20(2):139-54.
4. Sachs GS, Nierenberg AA, Calabrese JR, Marangell LB, Wisniewski SR, Gyulai L, et al. Effectiveness of adjunctive antidepressant treatment for bipolar depression. *N Engl J Med*. 2007;356(17):1711-22.
5. Calabrese JR, Guelfi JD, Perdrizet-Chevallier C. Agomelatine adjunctive therapy for acute bipolar depression: preliminary open data. *Bipolar Disord*. 2007;9(6):628-35.
6. McElroy SL, Weisler RH, Chang W, Olausson B, Paulsson B, Brecher M, et al. A double-blind, placebo-controlled study of quetiapine and paroxetine as monotherapy in adults with bipolar depression (EMBOLDEN II). *J Clin Psychiatry*. 2010;71(2):163-74.
7. Miklowitz DJ. Adjunctive psychotherapy for bipolar disorder: state of the evidence. *Am J Psychiatry*. 2008;165(11):1408-19.
8. Fountoulakis KN. An update of evidence-based treatment of bipolar depression: where do we stand? *Curr Opin Psychiatry*. 2010;23(1):19-24.
9. Ng F, Mammen OK, Wilting I, Sachs GS, Ferrier IN, Cassidy F, et al. The International Society for Bipolar Disorders (ISBD) consensus guidelines for the safety monitoring of bipolar disorder treatments. *Bipolar Disord*. 2009;11(6):559-95.
10. Hallam KT, Smith DI, Berk M. Differences between subjective and objective assessments of the utility of Electroconvulsive therapy in patients with bipolar and unipolar depression. *J Affect Disord*. 2009;112(1-3):212-8.
11. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, et al. So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med*. 2013;11:200.
12. Stertz L, Magalhaes PV, Kapczinski F. Is bipolar disorder an inflammatory condition? The relevance of microglial activation. *Curr Opin Psychiatry*. 2013;26(1):19-26.
13. Brietzke E, Stertz L, Fernandes BS, Kauer-Sant'anna M, Mascarenhas M, Escosteguy Vargas A, et al. Comparison of cytokine levels in depressed, manic and euthymic patients with bipolar disorder. *J Affect Disord*. 2009;116(3):214-7.
14. Kim YK, Jung HG, Myint AM, Kim H, Park SH. Imbalance between pro-inflammatory and anti-inflammatory cytokines in bipolar disorder. *J Affect Disord*. 2007;104(1-3):91-5.
15. Modabbernia A, Taslimi S, Brietzke E, Ashrafi M. Cytokine alterations in bipolar disorder: a meta-analysis of 30 studies. *Biol Psychiatry*. 2013;74(1):15-25.
16. Ng F, Berk M, Dean O, Bush AI. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. *Int J Neuropsychopharmacol*. 2008;11(6):851-76.
17. Andreatza AC, Kauer-Sant'anna M, Frey BN, Bond DJ, Kapczinski F, Young LT, et al. Oxidative stress markers in bipolar disorder: a meta-analysis. *J Affect Disord*. 2008;111(2-3):135-44.
18. Fernandes BS, Gama CS, Cereser KM, Yatham LN, Fries GR, Colpo G, et al. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis. *J Psychiatr Res*. 2011;45(8):995-1004.

19. Tramontina JF, Andrezza AC, Kauer-Sant'anna M, Stertz L, Goi J, Chiarani F, et al. Brain-derived neurotrophic factor serum levels before and after treatment for acute mania. *Neurosci Lett*. 2009;452(2):111-3.
20. Moutsatsou P, Tsoporis JN, Salpeas V, Bei E, Alevizos B, Anagnostara C, et al. Peripheral blood lymphocytes from patients with bipolar disorder demonstrate apoptosis and differential regulation of advanced glycation end products and S100B. *Clin Chem Lab Med*. 2014;52(7):999-1007.
21. Berk M, Kapczinski F, Andrezza AC, Dean OM, Giorlando F, Maes M, et al. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. *Neurosci Biobehav Rev*. 2011;35(3):804-17.
22. Berk M, Malhi GS, Gray LJ, Dean OM. The promise of N-acetylcysteine in neuropsychiatry. *Trends Pharmacol Sci*. 2013;34(3):167-77.
23. Chairungsrilerd N, Furukawa K, Ohta T, Nozoe S, Ohizumi Y. Histaminergic and serotonergic receptor blocking substances from the medicinal plant *Garcinia mangostana*. *Planta Med*. 1996;62(5):471-2.
24. Gutierrez-Orozco F, Chitchumroonchokchai C, Lesinski GB, Suksamrarn S, Failla ML. alpha-Mangostin: anti-inflammatory activity and metabolism by human cells. *J Agric Food Chem*. 2013;61(16):3891-900.
25. Nakatani K, Yamakuni T, Kondo N, Arakawa T, Oosawa K, Shimura S, et al. gamma-Mangostin inhibits inhibitor-kappaB kinase activity and decreases lipopolysaccharide-induced cyclooxygenase-2 gene expression in C6 rat glioma cells. *Mol Pharmacol*. 2004;66(3):667-74.
26. Sanchez-Perez Y, Morales-Barcenas R, Garcia-Cuellar CM, Lopez-Marure R, Calderon-Oliver M, Pedraza-Chaverri J, et al. The alpha-mangostin prevention on cisplatin-induced apoptotic death in LLC-PK1 cells is associated to an inhibition of ROS production and p53 induction. *Chem Biol Interact*. 2010;188(1):144-50.
27. Pedraza-Chaverri J, Reyes-Fermin LM, Nolasco-Amaya EG, Orozco-Ibarra M, Medina-Campos ON, Gonzalez-Cuahutencos O, et al. ROS scavenging capacity and neuroprotective effect of alpha-mangostin against 3-nitropropionic acid in cerebellar granule neurons. *Exp Toxicol Pathol*. 2009;61(5):491-501.
28. Boeckeler K, Adley K, Xu X, Jenkins A, Jin T, Williams RS. The neuroprotective agent, valproic acid, regulates the mitogen-activated protein kinase pathway through modulation of protein kinase A signalling in *Dictyostelium discoideum*. *Eur J Cell Biol*. 2006;85(9-10):1047-57.
29. Gutierrez-Orozco F, Failla ML. Biological activities and bioavailability of mangosteen xanthenes: a critical review of the current evidence. *Nutrients*. 2013;5(8):3163-83.
30. Khaw KY, Choi SB, Tan SC, Wahab HA, Chan KL, Murugaiyah V. Prenylated xanthenes from mangosteen as promising cholinesterase inhibitors and their molecular docking studies. *Phytomedicine : international journal of phytotherapy and phytopharmacology*. 2014;21(11):1303-9.
31. Chin YW, Kinghorn AD. Structural Characterization, Biological Effects, and Synthetic Studies on Xanthenes from Mangosteen (*Garcinia mangostana*), a Popular Botanical Dietary Supplement. *Mini Rev Org Chem*. 2008;5(4):355-64.
32. Malawska B. New anticonvulsant agents. *Curr Top Med Chem*. 2005;5(1):69-85.
33. Segal J, Berk M, Brook S. Risperidone compared with both lithium and haloperidol in mania: a double-blind randomized controlled trial. *Clin Neuropharmacol*. 1998;21(3):176-80.
34. Berk M, Ichim L, Brook S. Olanzapine compared to lithium in mania: a double-blind randomized controlled trial. *Int Clin Psychopharmacol*. 1999;14(6):339-43.
35. Berk M, Nierenberg AA. Three Paths to Drug Discovery in Psychiatry. *Am J Psychiatry*. In press.