

# **Effects of Polyphenols-rich Tropical Fruits on Mental Health Protection – A Clinical Trial Among Middle Aged Women**

## **Introduction**

Poor mental health has been identified as one of the main health problems among middle aged adults (National Mental Health Registry 2003). Polyphenols, which are a major antioxidants source, have been proven to be beneficial towards the prevention of various diseases including cancer and diabetes and more recently in mental health. The aim of this research was to determine the effectiveness of polyphenols-rich tropical fruit juice TP 3-in-1™ juice towards the improvement of mental health status.

## **Rationale of the Study**

Malaysia is a rapidly developing country with great improvement in her healthcare systems since independence. This will subsequently increases the life expectancy among Malaysians and the number of older adults is expected to increase in the near future. Poor mental health and cognitive status which may lead to other serious health conditions are the most feared part of getting old. Many studies have shown that signs of decreasing mental health and cognitive status start from the middle age, however, most cross-sectional and clinical trials in this field mainly focus the elderly population, without giving adequate attention for the middle-aged adults. To the best of our knowledge, there are still no clinical trial which studies the effects of polyphenols on mental health and cognitive status conducted among the middle-aged adults. Early intervention since the middle age will prevent early deterioration in mental health and cognitive status and and promote healthy aging and better quality of life during old age.

In addition, middle-aged adults are highly driven individuals and focus on the developments of their family and careers. They also contribute greatly towards the advancement of the economy of a nation. Therefore, many aspects will be negatively affected if their health, which includes mental health and cognitive status, are not in excellent conditions. Additionally, due to their high familial and career commitments, this group may be at risk of developing psychological stress, which may affect their health. Due to this, there is an urgent to conduct a comprehensive research in this group to maintain good health.

Currently, most researchers refer to the National Health and Morbidity Survey (NHMS) 2015 to study the prevalence and risk factors of poor mental health among the middle-aged adults

(Institute of Public Health 2015). However, this report only includes the general mental health and does not study the various parameters of mental health such as somatic symptoms, anxiety and insomnia, social dysfunction and severe depression. These parameters are important to be studied as previous researches have shown that it is common during aging (Singh-Manoux et al. 2012). Additionally, the report does not include dietary risk factors, which are main determinants in the development of poor mental health and cognitive status during middle-age. Determination of the risk factors will assist in the identification of middle-age adults with the risk to develop these conditions, and therefore, early medical intervention can be provided to this group.

Metabolomics is incorporated in this research in order to study the biochemical pathways which are responsible for the improvement of mental health and cognitive status after the supplementation of polyphenols-rich tropical juice. This is important as previous researches mostly focus on the roles of polyphenols in reducing oxidative stress, however, as the field of mental health and cognitive is wide and involve various factors, metabolomics analysis might be able in discovering other responsible mechanisms. Subsequently, this may contribute towards the nutritional and pharmacological field of mental health and cognitive health. Functional food and drugs which target these mechanisms can be developed in order to maintain excellent mental health and cognitive status during aging.

Fruits rich in polyphenols have received attention in its ability to attenuate this problem. However, most fruit in the researches are from the Western countries, like the berries, which may not be easily found in Malaysia. Tropical fruit which has equal or better level of polyphenols, such as pomegranate, guava and roselle, should be considered for researches regarding poor mental health and cognitive status during aging as it is more easily accessible in Malaysia. Thus, this clinical trial was conducted to investigate the effects of polyphenols-rich tropical juice on the mental health and cognitive status among middle aged adults, through a randomised, placebo-controlled design. Findings of this study are important for initiating an early intervention programme against poor mental health and cognitive status, and prevent the progression towards more serious conditions at old age.

### **Study Design**

This randomised, placebo-controlled clinical trial was conducted to evaluate the efficacy of polyphenols-rich TP 3-in-1™ juice in improving mental health and cognitive status among

middle aged adults. Additionally, this clinical trial would like to determine the effects of polyphenols-rich TP 3-in-1™ juice towards the improvement of health parameters, such as blood pressure, fasting blood glucose, lipid profile and lipid peroxidation. Metabolomic profiling was also included determine the effect of the juice supplementation on metabolites profiles.

### **Research Question**

Does the supplementation of polyphenols-rich TP 3-in-1™ tropical juice improves the mental health and cognitive status among middle-aged adults?

### **Research Objectives**

#### *1. General Objective*

To study the effect of supplementation of polyphenols-rich TP 3-in-1™ tropical fruit juice intervention programme to improve mental health and cognitive status among middle-aged adults (aged 45 to 59 years)

#### *2. Specific Objectives*

- a. To evaluate the effect of supplementation of polyphenols-rich TP 3-in-1™ tropical fruit juice in improving mental health and cognitive status among middle-aged adults
- b. To determine the effect of polyphenols-rich TP 3-in-1™ tropical fruit juice supplementation on health parameters: blood pressure, fasting blood glucose and lipid profile and lipid peroxidation among middle-aged adults
- c. To identify the effect of polyphenols-rich TP 3-in-1™ tropical fruit juice supplementation on metabolites profiles through metabolomic approach

### **Research Hypothesis**

1. Supplementation of polyphenols-rich TP 3-in-1™ tropical fruit juice improves mental health and cognitive status among middle-aged adults (45 to 59 years old)
2. Supplementation of polyphenols-rich TP 3-in-1™ tropical fruit juice is able to improve health parameters: blood pressure, fasting blood glucose and lipid profile and lipid peroxidation among middle-aged adults
3. Supplementation of polyphenols-rich TP 3-in-1™ tropical fruit juice shows differences in the metabolomics profiles

## **Methodology**

### *1. Pre-Testing of Questionnaires*

Prior to this clinical trial, pre-testing of questionnaires was conducted among five middle-aged individuals living in Flat Seri Sabah 3A. The aim of the pre-testing was to evaluate the understanding of the individuals about the questionnaires. In addition, the pre-testing was also conducted to familiarise the researchers with all the protocols and also to identify any problems in completing the questionnaires.

### *2. Study Ethics*

Research proposal was presented to the Medical Research Ethics Committee UKM on 13th June 2013 and subsequently, research protocols of this study were approved by the committee. The protocols of the study were explained to subjects using Information Sheet for Subjects. Subsequently, consent forms were completed by the subjects if they agreed to participate in this research.

### *3. Study Location*

Subjects were recruited from a housing flats for low- to middle-income population situated in Kuala Lumpur. Data collection was conducted at the Clinical Trial Ward, UKM Medical Centre. This clinical trial involved supplementation of polyphenols-rich TP 3-in-1™ juice which had obtained trade secret protection from Centre for Collaborative Innovation, UKM on 16th March 2016. The ward was opened in year 2000 to give assistance to investigators to conduct clinical trials. A total of one head nurse and two staff nurses working in the Clinical Trial Ward are ready to provide clinical services, such as blood withdrawing. Facilities such as centrifuge machine and freezer are also available in the ward.

### *4. Supplementation*

The subjects were supplemented with the juice or placebo three days in a week. The placebo beverage contained no juice or natural polyphenol but was formulated to look and taste like TP 3-in-1™ juice and to provide the same energy content. After the subjects had agreed and signed the consent form, the juice and placebo beverages were supplied on a weekly basis to the subjects' houses. Time of consumption and methods of storage of beverages were explained to subjects during each weekly meeting.

Preparation of both beverages were conducted once a week in the Dietetics Laboratory. The juice was then packed in white opaque bottles and labelled with the subjects' names, date of consumption, methods of storage and the researchers' phone number and stored in -4 to +4 °C temperature prior to distribution to the subjects. The bottles were then packaged in black bag before it were given to the subjects. Subjects were reminded to store the beverages in their refrigerator and to take their juice on the assigned date. Subjects were also reminded to restrict the consumption of polyphenols-rich food throughout the study period.

During the days of consumption, a total of 1500 ml of TP 3-in-1™ juice was taken in equal amount (500 ml) before three mealtime (breakfast, lunch and dinner). The supplementation period was 10 weeks. Subjects were instructed not to discard unfinished beverages from the bottles. The researchers collected the old bottles with any unfinished drinks the following week, together with sending new stock of beverages.

The dosage of TP 3-in-1™ juice was based on previous clinical trial by Krikorian et al. (2010) among elderly individuals with impaired cognitive. In the study, subjects were supplemented with 532 ml of Welsh's Concord grape juice every day, which provided 203.8 mg of anthocyanins. Previous researches have shown that anthocyanins is the main component of flavonoids that is responsible for the improvement of cognitive status related to aging (Krikorian et al. 2009).

## 5. *Study Outcomes*

Data collection was conducted three times throughout this clinical trial, starting from baseline, week 5 and week 10 of supplementation. Data taken include anthropometric measurement, blood pressure assessment, personal, social, health and lifestyle information, dietary assessment, General Health Questionnaire-28 (GHQ-28) for mental health assessments and Rey's Auditory Verbal Learning Test (RAVLT), Digit Span and Comprehensive Trail Making Test (CTMT) for cognitive assessments.

### a. Blood Analysis

A total of 15 ml of venous blood was drawn in ethylenediaminetetraacetic acid (EDTA), serum separating and sodium fluoride tubes. The blood was drawn by nurses at the Clinical Trial Ward after overnight fast by the subjects. The blood samples were then sent to Quantum

Dynamics Sdn. Bhd. for quick analysis (within 3 hours) of blood lipid profile, glucose, HbA1C, vitamin B12, folate and homocysteine.

#### b. Lipid Peroxidation

Quantification of lipid peroxidation is essential to assess oxidative stress in pathophysiological processes. The end products of lipid peroxidation are reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), as natural bi-products (ABCAM 2015). The free MDA present in the sample reacts with thiobarbituric acid (TBA) to generate a MDA-TBA adduct. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage.

For the analysis of lipid peroxidation, a total of 5 ml of venous blood drawn into EDTA tubes was centrifuged at 3,000 rpm for 10 minutes at 4 °C for plasma separation. The plasma was transferred into multiple aliquots of 1.5 ml microcentrifuge tubes. It was then stored at -30 °C and thawed only prior to the analysis. Analysis of lipid peroxidation was conducted using a kit by ABCAM (USA). The kit was kept at -20 °C immediately after it was received from the supplier. The kit consisted of phosphotungstic acid solution, butylated hydroxytoluene (BHT) (100X), thiobarbituric acid (TBA) solution and malondialdehyde (MDA) standard (4.17 M). Microplate spectrophotometer was used for the measurement of absorption. All components of the kit were thawed to room temperature prior to analysis.

#### c. Proton (1H) NMR Metabolomics

The protocol of NMR analysis for human urine samples by Beckonert et al. (2007) was used in this metabolomics profiling experiment.

##### Collection of urine samples

Urine samples were collected in urine collection bottles for metabolomics analysis. As the first void urine was rather more variable than the subsequent voids, the second void urine was used in this study (Lenz et al. 2003). Urine samples were collected in sterile 60 ml urine collection bottles and later transferred into multiple aliquots of 4 ml in 5 ml sterile screw cap centrifuge tubes. A total of 10 µl of sodium azide (0.1% wt/vol) was added to the aliquots of urine samples. Preservative sodium azide was used to prevent bacterial growth in the stored urine. The centrifuge tubes were sealed tightly and stored at -80 °C until analysis.

### Preparation of NMR samples

Urine samples were thawed and equilibrated to room temperature prior to the analysis. Once thawed, the samples were centrifuged for 3000 rpm for 15 minutes. The supernatant was collected and used in subsequent analysis.

A total of 400 µl of the urine sample was then mixed with 200 µl of phosphate buffer (pH 7.4) in a microcentrifuge tube, and later centrifuged at 12,000g for 5 minutes at 4 °C. A total of 550 µl of the samples was transferred into a 5 mm NMR tube.

### Proton (<sup>1</sup>H) NMR Acquisition

The NMR experiments were acquired at constant temperature of 32.2 °C with acquisition time of 7 minutes 35 seconds. The samples were loaded into the autosampler and the temperature was calibrated and kept constant before each experiment. Subsequently, the reference signal of the deuterated solvent was locked and the magnetic field was homogenized (shimming). In order to observe the dynamic range of metabolites concentrations efficiently, the water signal was suppressed by running 1D NOESY-presat experiments. The number of scans was set at 64 for the urine samples.

### **Statistical Analysis**

In order to analysis the difference between treatment and placebo groups in Phase 2, independent student t-test and chi square test were conducted with the result presented as means and standard deviation with 95% confidence interval.

In addition, repeated measures analysis of variance (ANOVA) was carried out to determine the effects of polyphenols rich TP 3-in-1™ juice consumption at baseline, week 5 and week 10 (time effect), group effect and interaction effect.

### **References**

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