ABSTRACT

Depression is a major human scourge. It is responsible for more "years lost" to disability than any other condition worldwide. To our disguise even in 21st century it is considered a taboo in our society to talk and seek professional help for it, the result being, rise in number of depression case among youth. Surprisingly, the majority of the population is unaware that they are prey to this severe condition, which is a direct cousin to a number of other lethal diseases. The need of the hour is lifestyle modification habits that have no societal repercussions in order to preserve and protect mental stability among young people. Various studies have revealed the secrets of our second brain viz. our gut, and therein lies the solution to mental health management: ***'get the right gut bacteria for your brain'.*** Gut microbes have a crucial role in brain development. Microbiota, in particular, influence the development of brain areas involved in stress response and the management of stress related disorders like anxiety and depression. To grasp the significance of the microbiome, it's necessary to first comprehend that the microbiome is assumed to connect directly with the brain via the Gut-Brain axis. Prebiotics, probiotics and fermented beverages provide exciting avenues for the modification of gut microflora into a positive balance. In view of this insight, doctoral work entitled **'Role of Fructooligosaccharide, Buttermilk and Biogenic metabolites released from fermented beverage (Ambil) as a communicator between gut and brain'** was planned and executed in eight phases:

Phase I of the study involved quantification of biogenic metabolites; β Casomorphin and Casoxin c in ambil using High Performance Liquid Chromatography. In milk, biologically active peptides are found in an inactive form inside the sequence of precursor molecules, which are released during fermentation. Ambil is an indigenous cereal buttermilk-based fermented beverage whose bacterial bionomics has been strengthened by adding prebiotic fructooligosaccharide (FOS). According to the HPLC graph projected, percentage of biogenic metabolites Casoxin C and ß Casomorphin in ambil was 21.3mg/kg and 6.2 mg/kg respectively.

Phase II was designed to gain insight into the intriguingly increased presence of depression among youth. Subjects aged 19-30 years who gave the duly filled and signed informed consent to fill the questionnaire and Beck's Depression Inventory (BDI) were

screened using a purposive sampling procedure from The Faculty of Family and Community Sciences, The MS University of Baroda, Gujarat. BDI was administered to 683 subjects to determine the presence of depression. Based on the scores subjects were classified into the normal, mild, borderline clinical, moderate, and severe categories. Participants were further oriented regarding the objective and the implications of the study before assessment. According to the assessment, a considerable percentage of university students (53.85%) scored between 11 and 30, putting them in the mild to moderately depressed category. Nearly 5% of people had severe depression and were completely ignorant of it. Highly significant difference (p value< 0.001) was observed in the category of depression with regard to major religion studied. High percentage of Muslim population (60.88%) were mild to moderately depressed followed by Christians and Hindus. Education didn't make any significant difference in terms of depression severity. Around 54% students in mild to moderate depression category were pursuing graduation. Significant difference (p value< 0.001) was observed between depression severity and family structure. People belonging to nuclear family (74.37%) reported to be more depressed than those who dwell in joint and extended joint families. Monthly per capita family income showed highly significant association with the depression status, (p value < 0.001).

The dietary profile of the subjects showed significant difference (p value ≤ 0.001) between severity of depression with respect to energy and macronutrient consumption. Mean energy, carbohydrate and protein consumption of mild to moderate and severely depressed subjects was less than RDA by 18.99%, 21.66%, and 8.26%; and 27.87%, 26.33%, and 15.21% respectively. Significant difference (p value ≤ 0.001) was observed between fat and depression status. However, fat consumption was relatively higher than RDA in all the subjects irrespective of depression severity. Consumption of the micronutrients assessed found to be lower than RDA in all the subjects irrespective of their depression status. Moreover, less consumption of micronutrients was observed in subjects experiencing severe depression when compared to normal. Statistically significant association between consumption of calcium (p value ≤ 0.05), magnesium (p value ≤ 0.05) and zinc (p value ≤ 0.001) and non-significant association with iron, omega 3 and omega 6 were observed with degree of depression. Mean intake of omega 3 and omega 6 was lower in severely depressed subjects (0.5 gm and 1.49 gm) than mild to moderately depressed (0.51gm and 1.53gm) and normal (0.71 and

2.12). Frequency of probiotic food consumption was assessed among subjects with mild to moderate depression. It was also considered whether they took pre/probiotic supplements as part of their everyday diet or as a kind of treatment. Subjects who reported taking pre/probiotics on a regular basis were excluded from all intervention stages. The screened patients' medical history was recorded using previously available health data and a questionnaire. Individuals with any serious ailment were not eligible to participate in the interventions. As depicted by study's result there is a direct link between depression and gastrointestinal motility (p value< 0.001). Severe constipation was reported by subjects falling in severe depression category (18.75%), whereas 12.5 % of subjects falling in mild- moderate depression zone and only 2% people among normal population reported constipation. Gut microbiota manipulation is crucial for the development of improved brain functions. The other phases of the study provide basis to Gut -Brain Axis. Using systemized random sampling taking into account the inclusion and exclusion criteria, subjects were segregated into five groups of 30 individuals each which received different supplementations.

In **Phase III, IV, V** and **VI** participants were intervened with 200 ml ambil (prebiotic added buttermilk based fermented beverage), 10 ml FOS, 200 ml fresh buttermilk and 200 ml tetra packed buttermilk respectively for a period of 45 days. Compliance was monitored with appropriate follow ups using daily SMS reminders and phone calls fortnightly. Specially designed calendar was distributed to all the participants to document daily consumption of supplements and unusual symptoms or side effects if observed. Enumeration of Gut macroflora (Ramona et al., 2001) with respect to *Lactic acid bacteria*, *Bifidobacterium*, and *E. coli*, depression status using Beck's Depression Inventory, and serum cortisol levels by Chemi Luminescent Immuno assay, in ROCHE machine were determined pre- and post-intervention trials. Ambil supplementation resulted in significant decrease of mean depression scores, and fecal log count of E. coli by 46.45%, and 2.88%. respectively. Drop in serum cortisol levels was observed by 6.56%. Significant improvement in colonization of *Lactobacillus* and *Bifidobacterium* was seen by 10.05% and 36.15%, respectively. Upon FOS supplementation significant decrease in mean depression scores and log count of *E. coli* was observed by 18.69% and 3.72 % respectively. Experiential increase in the colonization of *Lactobacillus and Bifidobacterium* was seen by 6.8% and 6.13% which was highly significant. Experimental group which received fresh buttermilk showed reduction in mean

depression scores and log count of *E. coli* by 14.21% and 2.71%, respectively. The gut colonisation with *Lactobacillus* and *Bifidobacterium* increased by 5.28 % and 4.51%, respectively. Intervention trials with tetrapacked buttermilk resulted in significant reduction in mean depression scores and log count of *E. coli* by 13.43% and 1.58%, respectively. A non-significant reduction in serum cortisol levels was seen by 7.38%. The colonisation of the gut with *Lactobacillus* and *Bifidobacterium* increased by 5.47% and 5.68% respectively. There were no significant differences in any of the parameters assessed upon intervention with prebiotic and fermented beverages in the control group.

In **Phase VII** of this study, the analysis was made to figure out the most effective supplementation in managing depression, serum cortisol levels and colonization of gut with good bacteria. Ambil proved out to be most effective supplementation measure in increasing the colonization of *Bifidobacterium* and *Lactic acid bacteria* followed by FOS, tetra packed buttermilk and fresh buttermilk. Highest reduction in the count of pathogenic bacteria *E. coli* was reported on FOS supplementation followed by ambil, fresh buttermilk and tetra packed buttermilk. The most promising supplementation for lowering cortisol levels was tetra packaged buttermilk tailed by ambil, fresh buttermilk and FOS. However, differences observed were scientifically significant but statistically non-significant.

All the four supplements- ambil, FOS, buttermilk tetra packed and fresh have proved their potential in improving the count of good gut bacteria and, managing depression. However, ambil proved out to be the most effective intervention followed by FOS, fresh buttermilk and tetra packaged buttermilk. in reducing mild to moderate depression. This may be due to the presence of prebiotic and release of biogenic metabolites during fermentation process in the beverage.

Phase VIII was incorporated into the study with the goal of raising public awareness about depression and how to deal with it. The software ‘Animaker' was used to create the animated film as an Information Education and Communication material.

This research has elicited positive association between increased colonization of good gut bacteria and improvement in depression status, proving the signaling between gut and brain. Maintaining the right gut bacteria is the key to good mental health.