

## CHAPTER 7

### SUMMARY AND CONCLUSION

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Depression is one of the leading diagnostic categories that constitute common mental disorders. Latest factsheet of WHO published in 2020 states 4.4% of the world population, suffer from depression.

Therefore, combating such a dreaded issue is an uphill task, needing lifestyle modification to strengthen mind's stability. Gut microflora could be used as a decipherable mode to understand and manage mental health. The importance of the microbiome on depressive state lies in the fact that these microbes are thought to communicate directly with the brain through the so-called Gut-Brain axis. FOS is considered to be a "true prebiotic"- essential nourishment for human gut. Milk based fermented beverages are well documented for their probiotic profile and prebiotic added fermented beverages have accounted for the presence of biogenic metabolites by the action of pro and prebiotics on milk protein casein.

In view of stated rationale present 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 and can be summarized with certain conclusion in following eight phases:

**PHASE -I:** *Quantification of Biogenic metabolites (B Casomorphin and Casoxin c) in ambil using High Performance Liquid Chromatography.*

**PHASE -II:** *Snap -Shoting the presence of mild to moderate depression among the subjects in the age group of 19-30 in the Faculty of Family and Community Sciences the Maharaja Sayajirao university of Baroda, Vadodara*

**PHASE -III:** *Impact evaluation of intervention trials with ambil (prebiotic enriched fermented beverage) on the fecal microbial counts (Lactobacillus, Bifidobacteria and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.*

**PHASE -IV:** *Impact evaluation of intervention trials with fructooligosaccharide on the fecal microbial counts (Lactobacillus, Bifidobacteria and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.*

**PHASE -V:** *Impact evaluation of intervention trials with fresh buttermilk on the fecal microbial counts (Lactobacillus, Bifidobacteria and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.*

**PHASE -VI:** *Impact evaluation of intervention trials with tetrapacked buttermilk on the fecal microbial counts (Lactobacillus, Bifidobacteria and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.*

**PHASE -VII:** *Evaluating which intervention had highest impact in reversing depression scores, cortisol levels and modulating gut microbiota composition.*

**PHASE -VIII:** *Developing an audio-visual animated film as Information Education Communication material to make general people aware about increasing trend of depression and how to cope up with it.*

**6.1. Phase I** of the research was undertaken to quantify the biogenic metabolites (B Casomorphin and Casoxin c) in ambil using High Performance Liquid Chromatography.

**Salient findings of Phase I were:**

- † Retention time of biogenic metabolites Casoxin C and  $\beta$  Casomorphin standards and samples as projected on HPLC graph was 15.24 and 21.03 respectively.
- † Percentage area covered by standards of Casoxin C and  $\beta$  Casomorphin was 54.43% and 32.32% respectively.
- † Percentage area covered by Casoxin C and  $\beta$  Casomorphin in analysed fermented beverage was 27.96 % and 17.60 % respectively.

- † Prebiotic enriched buttermilk based fermented beverage (ambil) reported the presence of 21.3 mg /kg of Casoxin C and 6.2mg/kg of  $\beta$  Casomorphin.

**Results of phase I clearly depicts the presence of Casoxin C and  $\beta$  Casomorphin in prebiotic added milk based fermented beverage (ambil). The two biogenic metabolites are known for their values in improving mental health conditions.**

**6.2. Phase II** of the research was undertaken to snap -shot the presence of mild to moderate depression. It was a prospective study in which a cross sectional design was used where in 683 subjects in the age group of 19-30 from the Faculty of Family and Community Sciences the Maharaja Sayajirao university of Baroda were enrolled. Beck's Depression Inventory was used to screen the subjects for depression. Background information, morbidity profile, defecation pattern, food and exercise habits, macro and micro nutrient intakes, and frequency of consumption of pro/prebiotic foods and supplements was accounted using a pretested structured questionnaire. Chi square test and Pearson's correlation was used to analyze and interpret the results of Phase II.

**Salient findings of Phase II were:**

- † As per the scores obtained through Beck's Depression Inventory, high percentage (53.8%) of screened subjects were suffering from mild to moderate depression. Less than fifty percent (41.43%) fell under normal category while 4.68% reported to be suffering from severe depression.
- † High percentage of muslim population (60.88%) were mild to moderately depressed followed by christians and hindus.
- † Education level of the subject didn't make any significant difference in terms of depression severity.
- † 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.

- † The dietary profile of the subjects showed significant difference ( $p$  value  $\leq 0.001$ ) 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  $\leq 0.001$ ) 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. Statistically significant difference between consumption of calcium ( $p$  value  $\leq 0.05$ ), magnesium ( $p$  value  $\leq 0.05$ ) and zinc ( $p$  value  $\leq 0.001$ ) were observed with degree of depression.
- † Subjects falling in the mild to moderate depression category were assessed for the frequency of milk based pre/probiotics. Frequent consumption was reported by 25% of the subjects while 63% subjects reported less consumption.
- † Type of diet has shown highly significant relation ( $p$  value  $\leq 0.001$ ) with depression severity. Vegetarians reported to be more severely depressed (43.75%) than ovo- lacto vegetarians (37.5%) and non-vegetarians (18.75%).
- † Frequency of consumption of tea/ coffee didn't show any significant relation with depression. People falling in the category of severe depression reported frequent consumption of aerated beverage (25%) compared to mild to moderately depressed (14.94%).
- † Physical activities like yoga, breathing exercise, walking and jogging practices have shown significant association with severity of depression ( $p$  value  $\leq$

0.001). Of severely depressed and mild to moderately depressed people nearly 65% and 56% never practiced yoga or breathing exercises.

- † A highly significant positive association ( $p$  value  $< 0.001$ ) was observed between constipation and depression.
- † Statistically significant negative correlation of depression was seen with log count of *Lactobacillus* ( $p$  value  $< 0.001$ ). *Bifidobacterium* and serum cortisol showed non-significant negative correlation with depression. Statistically significant positive correlation of depression was seen with *E. coli* ( $p$  value  $< 0.001$ ), and constipation ( $p$  value  $< 0.001$ ). Serum cortisol showed non-significant negative correlation with log count of *Lactobacillus* and, positive correlation with log count of *E. coli* and constipation.
- † Fecal log count of *Lactobacillus* showed significant negative correlation with log count of *E. coli* ( $p$  value  $< 0.001$ ) and constipation ( $p$  value  $< 0.001$ ). A non-significant positive correlation was observed with log count of *Bifidobacterium*.
- † Log count of *Bifidobacterium* showed non-significant negative correlation with log count of *E. coli* and constipation. Log count of *E. coli* also showed to be positively associated with constipation ( $p$  value  $< 0.001$ ).

**Results of phase II clearly depicts that majority of the university subjects were mild to moderately depressed. Nearly five percent were suffering from severe depression, but they were unaware about it. This phase also dialogues the various aspects attached with depression among youth including age, gender, family type and income. Results also pointed that nutritional allowance should not be taken for granted when it comes to proper brain functioning. Data received highlights the role of both macro and micro nutrient consumption on one's mental status. Direct association of depression with intestinal motility and gut microbial profile was noted.**

*Systemized Random Sampling was used to divide the subjects into five groups.*

**6.3. Phase III** of the research was undertaken to determine the impact evaluation of intervention trials with ambil (prebiotic enriched fermented beverage) on the fecal microbial counts (*Lactobacillus*, *Bifidobacteria* and *Enteric* pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects. Previously mentioned methods were employed for biochemical, microbial estimations and statistical analysis.

**Salient findings of Phase III were:**

- † A significant decrease in mean depression scores ( $p \text{ value} \leq 0.001$ ) was observed post intervention (46.45%) with ambil.
- † The fecal log count of *Lactobacillus* and *Bifidobacterium* showed a significant increase ( $p \text{ value} \leq 0.001$ ) by 10.05% and 36.15% respectively and a significant reduction of 2.88% in *E. coli* was observed ( $p \text{ value} \leq 0.001$ ).
- † Statistically significant improvement in defecation profile ( $p \text{ value} \leq 0.001$ ) in terms of stool frequency was seen by 0.28%.
- † A non-significant reduction was seen in the levels of serum cortisol in the experimental group.

**Randomized clinical trial with ambil to mild to moderately depressed subjects concludes that this prebiotic added milk based fermented beverage significantly lowers depression scores and cortisol levels via the mechanism of improving gut health, proving the connection between gut- brain axis.**

**6.4. Phase IV** of the research was impact evaluation of intervention trials with fructooligosaccharide on the fecal microbial counts (*Lactobacillus*, *Bifidobacteria* and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.

**Salient findings of Phase IV were:**

- † Impact evaluation of fructooligosaccharide supplementation showed significant decrease ( $p$  value  $< 0.001$ ) in percentage of depression and log count of *E. coli* by 18.69% and 3.72% respectively.
- † Experiential Increase in colonization of *Lactobacillus* and *Bifidobacterium* was seen by 6.8% and 6.13% which was highly significant ( $p$  value  $< 0.001$ ).
- † Statistically significant reduction in constipation ( $p$  value  $\leq 0.05$ ) in terms of stool frequency was seen by 0.19%.
- † A non-significant difference was observed post supplementation in the parameters assessed in the control group.

**Introducing liquid FOS to mild to moderately depressed subjects augment the gut microflora to a positive balance by increasing the colonization of *Lactobacillus* and *Bifidobacterium* and reducing the count of *E. coli*. Depression scores and cortisol values were reduced subsequently. Which in turn claims FOS to be effective feed for managing mental health.**

**6.5. Phase V** of the research was impact evaluation of intervention trials with fresh buttermilk on the fecal microbial counts (*Lactobacillus*, *Bifidobacteria* and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.

**Salient findings of Phase V were:**

- † Supplementation with fresh buttermilk, brought significant diminution (p value <0.001) in the depression scores and log count of *E. coli* by 14.21 % and 2.71%.
- † Gut health improved with significant increase (p value <0.001) in colonization of *Lactobacillus* and *Bifidobacterium* by 5.28% and 4.51%.
- † No significant changes were seen in control group with respect to any of the parameters pre and post intervention.
- † A non-significant reduction in the levels of serum cortisol was seen upon supplementation and comparative analysis with controls.

**6.6. Phase VI** of the research was impact evaluation of intervention trials with tetrapacked buttermilk on the fecal microbial counts (*Lactobacillus*, *Bifidobacteria* and Enteric pathogens), depression status, serum cortisol and defecation *profile of mild to moderately depressed subjects*.

**Salient findings of Phase VI were:**

- † Tetra packed buttermilk supplementation brought significant decrease in the depression scores (p value <0.05) and log count of *E. coli* (p value <0.01) by 13.43% and 1.58% respectively.
- † Gut health improved (p value < 0.001) with significant increase in the colonization of *Lactobacillus* and *Bifidobacterium* by 5.47% and 5.68%.
- † A not significant decrease in the levels of serum cortisol was marked by 7.38%. No significant changes were seen in the control group with respect to any of the parameters *post intervention*.
- † Comparative analysis with control group revealed, significant difference in depression scores and *Lactobacillus* count (p value < 0.001) and highly



significant difference in the mean log count of *Bifidobacterium* (p value < 0.001).

**Increase in colonization of *Lactobacillus* and *Bifidobacterium* and decrease in *E. coli* was noticed on fecal microbial analysis post intervention by tetrapacked buttermilk. Highly significant changes in depression scores were also noted, but no significant changes were there in cortisol levels.**

**Buttermilk consumption both tetrapacked and fresh improved the gut bacterial profile in mild to moderately depressed subjects by increasing the count of good bacteria *Lactobacillus* and *Bifidobacterium* and decreasing the count of pathogenic *E. coli*. Efficacy of age-old fermented beverage ‘buttermilk’ in combating depression was elicited.**

**6.7. In Phase VII** of the research was *evaluating which intervention had highest impact in reversing depression scores, cortisol levels and modulating gut microbiota composition.*

**Salient findings of Phase VII were:**

- † Intervention trials with all the four food products viz. Ambil, FOS, tetra packed buttermilk and fresh buttermilk shifted the gut microbial colonization to positive balance and brought about significant changes in depression status.
- † Ambil was most effective in lowering depression followed by FOS, fresh buttermilk, and tetra packed buttermilk. Mean square difference within the groups and in between the group for depression status was highly significant (p value < 0.001).
- † Tetra packed buttermilk proved out to be most effective intervention in reducing cortisol levels tailed by ambil, fresh buttermilk and FOS. These differences were non-significant within and in between the experimental groups as interpreted by post hoc test.
- † Log count of *Lactic acid bacteria* increased highest in the group supplemented with ambil followed up by FOS, tetra packed buttermilk and fresh buttermilk.

Mean square difference between groups and within groups was significant at F value 3.26 and p value < 0.01.

- † Ambil proved out to be most effective supplementation measure in increasing the colonization of *Bifidobacterium* followed by FOS, tetra packed buttermilk and fresh buttermilk. The mean difference between the intervention groups and within groups showed high significance (p value < 0.001).
- † Statistically significant difference (p value < 0.05) was observed in reduction of *E. coli* upon supplementation with various psychobiotics. Highest reduction in the count of pathogenic bacteria *E. coli* was reported in group on FOS supplementation followed by ambil, fresh buttermilk and tetra packed buttermilk supplementation.

**Results of phase VII clearly depicts that intervention trials with all the four food products viz. Ambil, FOS, tetra packed buttermilk and fresh buttermilk shifted the gut microbial colonization to positive balance and brought about significant changes in depression status. Though, all the given supplements have proved their potential in strengthening gut brain axis- managing depression by improving gut profile, ambil proved out to be the most effective intervention in improving the count of good gut bacteria and thus, reversing depression this may be due to the presence of prebiotic and probable release of biogenic metabolites during fermentation process in the beverage. The current research evoked the goodness of consuming synbiotic over prebiotic and probiotic in depression management.**

**6.8. Phase VIII** of the research was developing an audio-visual aid as a means of IEC material to make general people aware about increasing trend of depression and how to cope up with it.

**In this phase Animated movie named ‘RESTART’ was developed using the software Animaker. The aim was to make general people aware every aspect of depression including symptoms, need to communicate with subject expert, the role of nutrition, exercise, and how to formulate new habits for the betterment of self.**