CHAPTER 6

Discussion

Mental health, particularly depression, is a worldwide concern and remains the most overlooked aspect of well-being. In India, one out of every seven adolescents meet the diagnostic criteria for a mental health disorder (Polanczyk et al., 2015), and one out of every twenty individuals suffer from depression (NMHS, 2016).

Early detection and prompt access to professional treatment can prevent mental health from getting worse. Various institutional-based studies point towards an increasing prevalence of depression among youth. Major concerns are negligence, social stigma and the fear of seeking professional help. A recent 2020 study identified 53 eligible studies which highlights the complex array of reluctant nature of youth for seeking help for mental health difficulties. Almost all studies (96%) reported barriers related to individual factors, such as limited mental health knowledge and broader perceptions of help-seeking. Studies (92%) pointed towards perceived social stigma and embarrassment while talking about mental health. Young people's impressions of therapeutic interaction with experts, such as perceived secrecy and the ability to trust an unfamiliar person, were captured in 68% of research. The fourth issue dealt with systemic and structural barriers and facilitators, such as the financial expenses of mental health care, logistical challenges, and the availability of professional assistance among 58% studies (Radez et al., 2020).

Looking at the current state of youth's relationship with their mental health, the research was designed to gain insight into the intriguingly increased frequency of depression among the youth (19-30 years) at the Faculty of Family and Community Sciences, The MS University of Baroda. The depression assessment was carried out by 683 subjects through self-assessed Beck’s Depression Inventory. The results revealed, a large number of subjects (53.85%) were mild to moderately depressed. Nearly five percent were suffering from severe depression, and they were unaware of it. Less than 50% (41.43%) of the total screened subjects indicated the absence of depression. These results are almost comparable to the data of other institutional based studies. Bhopal University conducted research using a theoretical Depressive Experiences Questionnaire that revealed 31% of students were depressed at the age of 21 (Alghamdi,

2015). A cross-sectional study conducted in Peoples University using Theoretical Depressive Experiences Questionnaire (TDEQ) reported the prevalence of depression among 31% of students (Sharma et al., 2015). Punjab University, Chandigarh assessed the mental health of 400 students from nine departments. The overall prevalence of depression, anxiety and stress came out to be 59.2%, 86.5% and 52.7% respectively (Singh et al., 2017). A cross-sectional survey conducted amongst 331 undergraduate students at college in Gujarat using the Patient Health Questionnaire reported the overall prevalence of depression to be 64% with the highest level of depression among first year students whereas, moderate to severe depression was found in 26.6% of students (Vankar et al., 2014). The rising trend in youth depression requires attention to both underlying issues and effective solutions to tackle the condition before it has severe societal impact.

The present chapter dialogues the various aspects attached to youth depression. Most subjects (94.87%) were in the age group of 19-25 and were females of which 53.5% reported to be mild to moderately depressed. The status of depression between the age group studied was significant (p value < 0.05). 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 (p value < 0.001) association with the depression status.

In line with the data, lies the results of research which correlated depression in 200 students of various educational institutes in Amritsar, Punjab using Patient Health Questionnaire. The majority of students were in the age group 18-24. The overall prevalence of depression noted was 16.5%. Out of all studied subjects, 81 (40.5%) were male and 119 (59.5%) were female. All of the subjects were unmarried (100%), the majority of whom lived in cities (77.5%), came from nuclear families (79.5%),

practiced Hinduism (57%), and belonged to a middle socioeconomic class (67%) (Kaur et al., 2014).

As revealed in a fantastic research study, meditation and breathing exercise done over 30 minutes a day for eight weeks modulate the density of the brain's grey region associated with learning, memory, and cognition. The hippocampus, an area of the brain associated with learning which tends to shrivel in size with age, also expands with meditation **(**Lardone et al., 2018). In the present research, physical activities like yoga, breathing exercise, walking and jogging have shown significant association with severity of depression (p value ≤ 0.001). Nearly 65% severely depressed and 56% of mild to moderately depressed people never practiced yoga or breathing exercises.

A study investigated the impact of a 100-day, 10,000 step program on signs of depression, anxiety and stress as well as general wellbeing using standardised psychological scales. The results indicated a small but consistent effect on all of these measures of mental health over the term of the program (Hallam et al., 2018). This effect appeared irrespective of whether a person reached the 10,000-step mark. According to the data obtained in the current study, 22.82% of mild to moderately depressed and 71% of severely depressed subjects never walk or jog as part of their daily activity. These results highlight, better mental health and wellbeing in people who jog or walk daily, indicating the efficacy and potential of basic exercise in good mental health.

Type of diet has shown highly significant relation (p value ≤ 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%). This was found to be statistically significant at p value < 0.001. A meta-analysis on vegetarianism and depressed mood published in the *Journal of Affective Disorders* (Ocklenburg and Borawski, 2021) compared data on individual scores in depression questionnaires from 13 different empirical studies. Overall, 8,057 vegetarians and 41,832 non-vegetarians were included in the analysis. The meta-analysis revealed a statistically significant difference between vegetarians and non-vegetarians, with vegetarians showing higher depression scores than non-vegetarians.

This research also opens an argument why 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 mental status. The dietary profile of the subjects showed significant association (p value ≤ 0.001) between severity of depression and energy and macronutrient (carbohydrate, protein and fat) consumption. Nutrient intake of subjects was compared to RDA for macronutrients and micronutrients for Indian Women, ICMR NIN 2020. Mean energy, carbohydrate and protein consumption of mild to moderate and severely depressed subjects was found to be less than RDA.

Carbohydrate rich foods trigger the secretion of brain chemicals serotonin and tryptophan. Therefore, a diet low in carbs interferes with the proper functioning of brain chemical messengers (Rao et al., 2008). Low-carbohydrate diet have also shown to induce metabolic depression (Ebrahimpour et al., 2019). Our results provide evidence of this fact, mean carbohydrate consumption of mild to moderate and severely depressed subjects was less than RDA by 21.66% and 23.33% respectively.

Similar findings were reported by D'Anci et al., 2009 where low carbohydrates were associated with greater risk of mood disorders including anxiety and depression. Protein is linked to neurotransmitter synthesis, a lack of amino acids in the diet has an effect on mood. A positive association exists between low protein intakes and the prevalence of mental illnesses (Nanri, 2015). The amino acid tyrosine is used to make the neurotransmitter dopamine, while tryptophan is used to make the neurotransmitter serotonin. There will be insufficient synthesis of the corresponding neurotransmitters if any of these two amino acids is deficient, which is linked to low mood and violence in patients. In the current study, the protein consumption of mild to moderately depressed and severely depressed subjects was lower than RDA by 15.89% and 29.09% respectively. Significant difference (p value ≤ 0.001) was also observed between fat and depression status. However, fat consumption was relatively higher than RDA in all the subjects irrespective of depression severity.

Findings support the importance of adequate consumption of zinc, selenium and magnesium in the promotion of mental health (Wang et al., 2018). Zinc is a trace element that is required for a variety of biochemical and physiological processes

connected to brain development and function (Jurowski et al., 2014). A meta-analysis of 17 observational studies discovered that blood zinc concentrations in depressed subjects were approximately 0.12 g/mL lower than in control subjects (Swardfager et al., 2013). Magnesium levels are critical for CNS function and may be linked to Alzheimer's disease, diabetes, stroke, hypertension, migraines, and attention deficit hyperactivity disorder (ADHD) (Gröber et al., 2015). In both animal and human investigations, a link between magnesium deficiency and depression has been established. Dietary magnesium deficiency alters gut microbiota and leads to depressive-like behaviour in mice (Winther et al., 2015). A randomised controlled trial of 60 people with depression and hypomagnesemia found that taking 500 mg magnesium oxide daily resulted in significant improvements in Beck Depression Inventory scores when compared to people with depression and hypomagnesemia who took a placebo (Rajizadeh et al., 2017). Looking at the data from the current result, consumption of micronutrients was also reportedly less in depressed individuals than normal. 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). Empirical evidence most strongly supports a positive association between micronutrient deficiency with mental health.

Studies have investigated patients with depression show indication of abnormal intestinal transit. Data from the National Health and Nutrition Examination Survey (NHANES) studied the relationship between depression and bowel habits. They analysed 31,191 adults from 2005–2016. The survey didn't include microbiome samples, only self-reported gastrointestinal symptoms and Depressive symptoms were measured using the Patient Health Questionnaire. Compared to those without depressive symptoms, those with moderate-to-severe depressive symptoms had elevated constipation (sometimes vs. never odds ratio = 2.76; 95% confidence interval = 2.11–3.62). Overall, those with gastrointestinal symptoms were significantly

more likely to have depressive symptoms (Eustis et al., 2021). In another Korean study chronic diarrhoea and constipation were more common in individuals with depression (22.3% and 18.6%, respectively) than in individuals with no depression (7.0% and 10.9%, respectively). In addition, they were more prevalent in individuals with anxiety (24.5% and 18.6%, respectively) than in individuals with no anxiety (9.1% and 12.7%, respectively) (Kim and Lim, 2021). The result of the present study also showed association between stool frequency and depression severity to be highly significant (p value < 0.001). Severe constipation was reported by subjects falling in severe depression category (18.75%) as compared to mild and moderately depressed (12.5 %).

Gut microbial modulation is the key to the establishment of better brain functionalities. Getting the right gut bacteria is one of the favorable options and the core connection for gut brain axis. Microbial fermentation transforms food substrates into more nutritionally and functionally dense products, resulting in functional microorganisms (probiotics), prebiotics (substrates that promote the growth of beneficial bacteria in the gut), and bioactive components (biogenic metabolites). These functional components have the ability to affect the gut microbiota, influence endotoxin translocation and subsequent immune activation, and increase host nutrition by acting biologically in the gastrointestinal system (Aslam et al., 2020). When the daily diet of subjects who obtained mild to moderate scores on Beck's Depression Inventory was looked upon in terms of consumption frequency of prebiotic, and fermented milk-based beverages; like *curd, buttermilk, yogurt, shrikhand, matho and lassi* it was clear subjects who frequently consumed such foods were less likely to have depression as compared to who consumed it less frequently. Other researches also support this algorithm, consumption of fermented milk prevents the onset of physical symptoms in medical students under academic examination stress (Kato-Kataoka et al., 2016). Adzuki bean (*Vigna angularis*) sprout fermented milk, has the potential to prevent or treat mild depression-like symptoms in mice (Wu et al., 2021).

Fermentation enhances the microflora in milk which act both as preservators and transformers as they have the power to amplify the bioavailability and activity of various biogenic metabolites which have therapeutic potential, the value of which is also associated with mental health (Selhub et al., 2014). The *Lactic acid bacteria* proteolytic system acts on casein present in milk and milk derivatives to supply

bioactive peptides (Clare and Swaisgood, 2000). These peptides have proved their worth in gut-brain communication, enhancing brain cognitive function (Dinan and Cryan, 2017). In a randomized, double-blind, placebo-controlled study, the consumption of fermented milk with multiple probiotic strains and prebiotic fibers has shown to improve constipation in Parkinson's patients (Barichella et al., 2016). In the present study we quantified the presence of bioactive peptides Casoxin C (21.3mg/kg) and ß Casomorphin (6.2mg/kg) in ambil (prebiotic added buttermilk based fermented beverage). According to research, beta-casomorphine (BCM) are the most well-studied myorelaxant peptides (Pessione and Cirrincione, 2016). Casoxin can be employed to counteract depression (Chiba and Yoshikawa, 1986). BCM7 has been detected and quantified in a number of pasteurized milk products. However, to date, specific enzymes contributing to the formation of BCM7 in dairy products are still unknown (Nguyen et al., 2015). Nguyen also identified β-casomorphin in yogurt. Systemic administration of low dose of bovine β-casomorphin-5 (1mg/kg i.p.) has been shown to improve the disturbance of learning and memory in mice (Sakaguchi et al., 2006). Products containing β-casomorphin also have the potential to improve intestinal health by preventing the adherence of pathogens to the intestinal surface and thus eliminating the onset of intestinal infections. Oral administration of this peptide at the rate of 0.1–100 μmol/L to rat pups enhanced the number of goblet cells and Paneth cells along the small intestine (Mann et al., 2017).

Despite the loss of probiotics due to the tetra packing of the ambil, we can still claim the product's goodness for health because released biogenic metabolites during fermentation makes the product directly beneficial without the need for live bacteria (Korhonen and Pihlanto, 2003). These molecules constitute a promising frontier for treating both stress-related behaviours such as anxiety and depression and season-related mood disorders (Pfluger et al., 2012). In general, these peptides are able to control the gut–brain axis at several levels, including gut–brain communication, brain cognitive function, and behaviour (Cryan and Dinan, 2012). It has been demonstrated that the biological effect is mediated by activation of serotonin and GABA receptors, causing release of endogenous serotonin, dopamine, and GABA (Mizushige et al., 2013).

To elicit the importance of establishing gut flora to a positive balance and asses their role in combating depression phases of the study were planned and implemented which focused on looking at the beneficial effect of intervention with psychobiotics -ambil, FOS, buttermilk fresh and tetrapacked to mild-moderately depressed subjects. The biochemical parameter involved was blood serum cortisol. Hypercortisolemia is an indicator for stress. Defecation profile with respect to constipation and fecal log count of gut floras *Lactobacillus* and *Biffidobacteria* and *E. coli* was studied before and after supplementation intervals.

Present research has shown that consumption of ambil having bioactive peptides casoxin C and ß casomorphin helps in the reduction of serum cortisol and depression scores. Supplementation of 200 ml of this beverage to 30 mild to moderately depressed subjects for 45 days resulted in a significant decrease in mean depression scores (p value ≤ 0.001) by 46.45%. The fecal log count of *Lactobacillus* and *Bifidobacterium* showed a significant increase (p value ≤ 0.001) by 10.05% and 36.15% respectively and a significant reduction of 2.88% in *E. coli* was observed (p value ≤ 0.001). Statistically significant improvement in defecation profile (p value ≤ 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 a double-blind study involving over 40 healthy male subjects, two groups were subjected to psychological and physical stress tests, with cortisol concentrations, heart rate, and blood pressure levels measured at specific intervals. Each of these three stress response markers were substantially lowered in the group taking the proprietary bioactive milk peptide compared to controls. In comparison, the milk peptide group experienced a net reduction in cortisol of 20.69%. In other words, the release of cortisol was kept under control by the *milk peptides*, limiting its detrimental effects (Messaoudi et al., 2011). Bioactive peptides have a role to play in mental health (Mohanty et al., 2016). A group of 60 women suffering from a constellation of stress-related problems were given 150 mg per day αs1-casein hydrolysate bioactive milk peptide compound. Compared to controls, after 30 days, significant improvements were observed in cognitive function 62.5% improvement, 16.3% over placebo (Kim et., al 2007). So, the presence of the bioactive peptides in ambil may be one probable reason for improved mental status.

The intestinal microbial community formulates the basis of crosstalk between the gut and brain. The idea that certain nutrients can augment the gut microflora existed before the definitions for such nutrients were proposed. Prebiotics, which can be described as the feed for the intestinal microbiota, hold the potential of increasing saccharolytic activity within the gut by acting as a catalyst in modulating gut microflora to a positive balance. Only 4g of fructooligosaccharide per day is sufficient to observe an increase in the gut bifidobacterial as pointed out by a Japanese study (Tuohy et al., 2003).

There is a growing body of evidence documenting the ability of prebiotics to normalize the dysbiosis microbiota associated with psychological disorders such as anxiety, depression, stress, autism, learning, and memory (Liu et al., 2105). The first report of the psychobiotics properties of prebiotics was examined on Sprague–Dawley rats. Prebiotic ingestion increased hippocampal BDNF expression and BDNF mRNA expression, which play an essential role in maintaining synaptic plasticity and optimal memory function (Li and Tsien, 2009). A supporting study found that rats fed the prebiotic during lactation had significantly improved maze-learning and object-recognition even one year later, indicating that prebiotic ingestion has very long-lasting benefits (Oliveros et al., 2016). In the present research 10 ml FOS supplementation for 45 days, increased the colonisation of *Lactobacillus* and *Bifidobacterium* by 6.8% and 6.13%, respectively, while a reduction in *E. coli* was seen by 3.72%, which was highly significant (p value ≤ 0.001). Hype surrounding the human microbiome influencing mental health stems from the belief that gut microbiota can modulate host brain function and behaviour via the microbiota-gut-brain axis. Present study demonstrated a significant decrease in mean depression scores by 18.69% (p value < 0.001) with the positive shift in microbial diversity. Consonant with our research results, there was a significant increase in mean log counts of beneficial gut microbiota with improvement in SAGE scores of the mild to moderate Alzheimer's subjects who were on FOS supplementation. Prebiotic Galactooligosaccharide consumption also lowered cortisol awakening reactivity and attenuated vigilance, which is a behavioural marker of anxiety and depression (Schmidt et al. 2015). Chen, 2017 through his work confirmed the secretion of neurotransmitter exerting effective memory improvements on consumption of Fructooligosaccharide. Supplementing 12 gm FOS to 65 obese adults for a period of 12 weeks resulted in a significant increase in *Lactic acid bacteria* and

Bifidobacterium by 14% and 10% respectively. With a 20% reduction in enteric pathogens (Sheth and Assudani, 2014). Data supported by a recent investigation carried on 80 participants reported FOS consumption increased the relative abundance of *Bifidobacterium* and *Lactobacillus* while its withdrawal led to its reduction. The positive impact of FOS-mediated increased bacterial diversity reinforces the role of prebiotics in conferring a positive effect on mental health (Tandon et al., 2019).

Buttermilk is usually processed by natural fermentation with lactic acid bacteria. The importance of this age-old Indian dairy beverage in defining gut bacteria cannot be overstated; numerous studies demonstrate its ability to prevent gastrointestinal infections by inhibiting bad bacteria such as *Helicobacter pylori.* The bacterial strains isolated from buttermilk used for supplementing the subjects of current research as identified by Hirdyani and Sheth, 2018 were *Bifidobacterium pseudocatenulatum, Bifidobacterium catenulatum, Bifidobacterium angulatum, Bifidobacterium kashiwanohense, Bifidobacterium ruminantium, Bifidobacterium merycicum, Bifidobacterium callitrichos, Bifidobacterium stercoris, Lactobacillus acidophilus, Lactobacillus acidophilus, Lactobacillus gallinarum, Lactobacillus gasseri, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus paracasei, Lactobacillus crispatus, and Lactobacillus crispatus.* In this research, the role of buttermilk consumption was assessed on gut profile and depression.

Buttermilk supplementation significantly reduced the log count of pathogenic bacteria *E.coli* by 2.71% (p value < 0.001), and gut health improved with a significant increase in colonization of *Lactobacillus* and *Bifidobacterium* by 5.28 % and 4.51% respectively (p value < 0.001). Subjects who consumed tetra packed buttermilk also showed significant increase (p value < 0.001) in log count of *Lactobacillus* and *Bifidobacterium* by 5.4% and 5.68% respectively and a reduction of *E. coli* by 1.58% was confirmed. Significant diminution in depression severity was observed on supplementation of buttermilk both fresh (p value < 0.001) and tetrapacked (p value < 0.05) by 14.21 % and 13.43 % respectively.

Interestingly, the concept of gut brain axis, improving mental health by modulating the count of good gut bacteria is gaining popularity. Prebiotics and fermented beverages holding nutraceutical properties have proven their worth in the establishment of better

microflora in the gut thus affecting brain functionalities. Increased count of good gut bacteria and decrease in count of pathogenic bacteria are the marker of healthy gut. This research results have also elicited the importance of prebiotics and fermented beverages in maintaining this count. We tried to make comparative analysis to figure out whether prebiotic or fermented beverage by themselves or a combination of duo -the goodness of fermented food modified with prebiotic, is better in modulating human psychiatry by triggering the colonization of good bacteria in the gut activating the channels of gut brain connection to bring about an improvement in the mental status of the individuals.

As previously discussed in the study, mild to moderately depressed subjects who were supplemented with prebiotic and fermented beverages showed remarkable increase in the count of good gut microflora *Lactobacillius* and *Biffidobacteria* while decreasing the count of pathogenic bacteria *E coli*.

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.

The positive role of dairy based fermented foods on mental health has been confirmed through various researches. Studies reported that consumption of fermented milk prevents the onset of physical symptoms in medical students under academic examination stress. Issue published in Psychiatry Research, August 2015 showed that college undergraduates who ate the most fermented foods, including yogurt were less likely to suffer from social anxiety, fear or depression (Kato-Kataoka et al., 2016). Consuming 200 ml of fermented milk per day for 12 weeks, significantly improved the Mini-Mental State Exam from 8.7 to 10.6 (Akbari et al., 2016). People who consumed yoghurt for two weeks had higher increases in serotonin levels (Kim et al., 2018). In a

randomized, double-blind, placebo-controlled study, the consumption of fermented milk with multiple probiotic strains and prebiotic fibers has been shown to improve constipation in Parkinson's patients (Barichella et al., 2016*).* In consonant with these research outcomes, lies our results. 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 (46.45%) followed by FOS (18.69%), fresh buttermilk (14.21%), and tetra packed buttermilk (13.43%). Improvement in mental health status following supplementation can be attributed towards the presence of prebiotic and biogenic metabolites released. Mean square difference within the groups and in between the group for depression status was highly significant (p value < 0.001).

The current research evoked the goodness of consuming prebiotic enriched fermented beverage over prebiotic and buttermilk in depression management.

Pearson's correlation was taken into account to compare the relativity of depression with various parameters. As expected, statistically significant negative corelation of depression was seen with log count of *Lactobacillius* (p value<0.001). *Biffidobacterium* showed non-significant negative corelation with depression. Statistically significant positive corelation of depression was seen with *E. coli* (p value<0.001), and constipation (p value<0.001).

In major depression and anxiety, hypercortisolaemia is one of the most reliable biological readouts (Gillespie and Nemeroff, 2005). However, results are inconsistent between saliva and blood testing methods. Most of the studies used salivary testing method and have shown reduced waking cortisol response and alters emotional bias on prebiotic and probiotic supplements. In a 30-day study, healthy volunteers with no previous depressive symptoms were given either probiotics or antidepressants. Those given probiotics showed reduced cortisol levels and improved self-reported psychological effects to a similar degree as participants administered Diazepam, a commonly used anti-anxiety medication (Lyte et al., 2011). Research performed in 2019 on prebiotics and probiotic consumption showed a decrease in levels of cortisol by 20% and 19% in experimental groups respectively (Kazemi et al., 2019). Prebiotic

intake reduces the waking on GOS supplement however, no effect was noticed on FOS supplementation (Schmidt et al., 2015).

We used the robust blood serum method for cortisol estimation, the results observed were

clinically significant in all the supplementation but not statistically significant. The contradiction in result of our study with review of literature may be due to the site of estimation as cortisol blood test measures all the cortisol contained in the blood, i.e., both the free/unbound cortisol and the cortisol bound to protein (cortisol binding globulin or CBG) while cortisol saliva test only measures the free cortisol. This can make the cortisol blood test result appear either high or low in the range compared to cortisol measured in saliva. Other more effective parameters can be taken into consideration like serotonin, dopamine, GABA. MRI scan can be a novel popular technique to pick up brain abnormalities in people with depression (Radiological Society of North America, 2019).

With an increase in the depression rate among young adults, there is a strong need to bridge the gap between communication and the lack of avenues for treatment. People still feel it a taboo to talk about mental health. Educating the community about depression is the need of the hour. It is spreading like an iceberg in our society. The major issue of concern is that people don't even realize that they are the sufferers. We made an attempt to create awareness about depression in the general population through the last phase of our research. An audio-visual animated movie was created in the hopes that the information- education -communication (IEC) developed as part of our study will be widely distributed among the general public, allowing people to develop a common understanding of depression, feel motivated to talk about it, and take precautionary measures ahead of time. Multimedia based communication has got better retention possibility compared to other methods. An animated movie developed using the software *Animaker* tried to cover every aspect of this disease, including symptoms, the need to communicate with subject expert, the role of nutrition and exercise and how to formulate new habits for betterment of self. The movie is named 'RESTART', which aims to ring the wake-up call to restart with new hope, new strength and new thoughts to overcome depression.