Supplementary materials

OsTea Randomized Control Trial and Translational Study Assessing the Efficacy of Herbal Teas on Bone Health and Quality of Life in a Population with Osteopenia: rooibos actions on melatonin and tulsi actions on quality of life
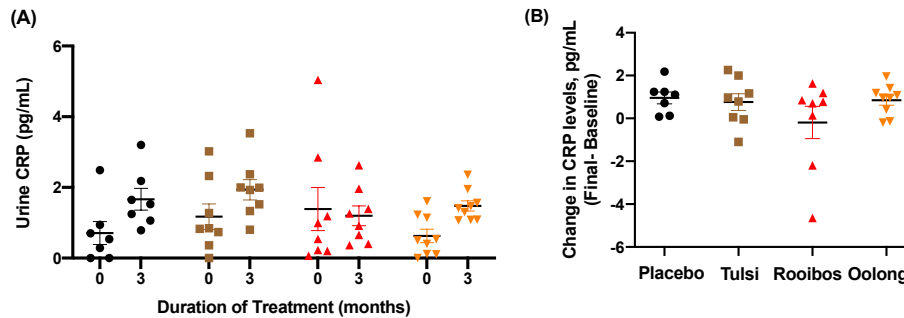

Fig. S1. Herbal teas effects on urinary CRP levels in all groups.

*Scatter plots represent (A) CRP levels at months 0 (baseline) and 3 (final) and (B) Changes from baseline to final after herbal tea consumption (placebo: black, tulsi: brown, rooibos: red, oolong: orange; n=8/9 per group). Mean changes from baseline to month 3 were compared among treatment groups using ANOVA followed Tukey multiple comparison test. Further Student's one-tailed t- test for all endpoints with Welch's correction were performed. The solid lines indicate the mean (± SEM) value for each group. *p ≤ 0.05 versus placebo at similar time point.*
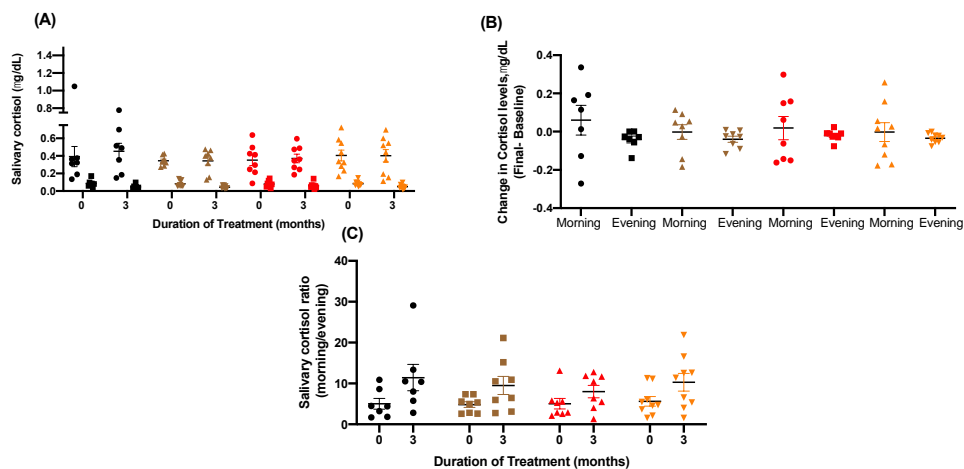

Fig. S2. Herbal teas effects on salivary cortisol (morning, evening) levels.

*Scatter plots represent (A) Morning and evening cortisol levels at months 0 (baseline) and 3 (final), (B) Changes from baseline to final after herbal tea consumption (placebo: black, tulsi: brown, rooibos: red, oolong: orange), and (C) morning to evening cortisol ratio; n=8/9 per group. Mean changes from baseline to month 3 were compared among treatment groups using ANOVA followed by Tukey multiple comparison test. Further Student's one-tailed t- test for all endpoints with Welch's correction were performed. The solid lines indicate the mean (± SEM) value for each group. *p ≤ 0.05 versus placebo at similar time point.*
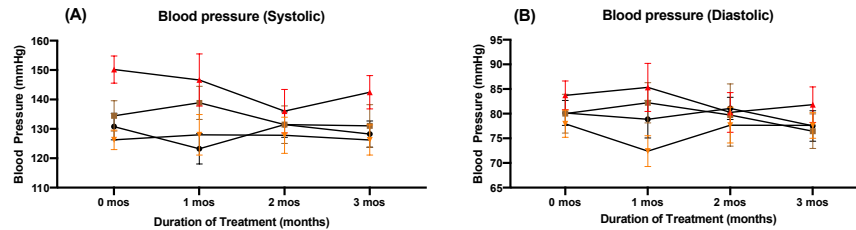
Fig. S3. Herbal teas effects on blood pressure.

(A) systolic blood pressure and (B) diastolic blood pressure measured at every month (placebo: black, tulsi: brown, rooibos: red, oolong: orange). Each point in the line graph represents the least square mean (± SEM). Mean changes were compared among treatment groups using ANOVA followed Tukey multiple comparison test. Further Student's one-tailed t- test for all endpoints with Welch's correction were performed. *p ≤ 0.05 versus placebo at similar time point.
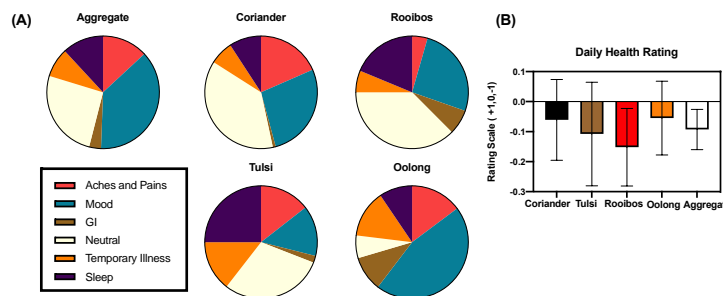

Fig. S4. Herbal teas effects on general well-being and compliance from daily diary logs analysis.

(A) Total diary comments made by the participants in each group throughout the study were stratified into six categories: sleep, mood, GI upset, general aches/pains, neutral and temporary illness as illustrated by the six segments in the pie diagram, (B) the average rating of all the comments for each tea.
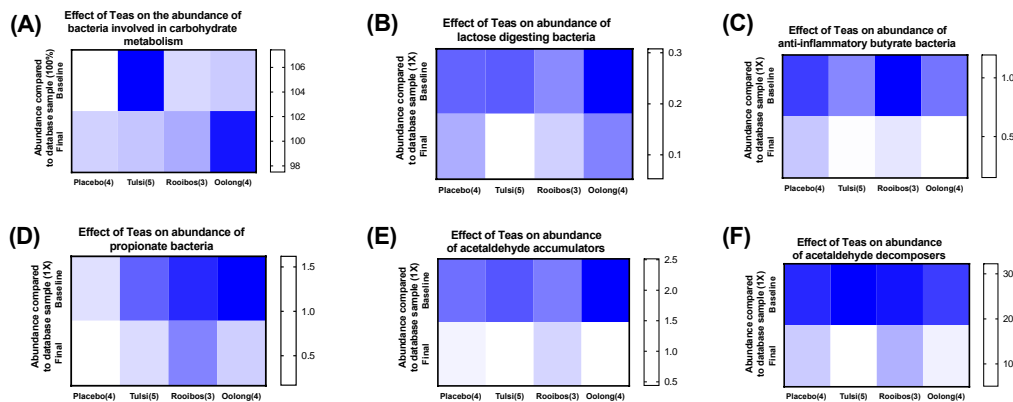

Fig. S5. Herbal teas effects on (A) carbohydrate metabolizing bacteria, (B) lactose (dietary disaccharide) digesting microbes, (C) butyrate (SCFAs from pyruvate metabolism [65]) producing bacteria, (D) propionate (SCFAs from pyruvate metabolism [65]) producing bacteria, (E) alcohol accumulator microbes (alcohol from pyruvate metabolism [65] to produce acetaldehyde), and (F) alcohol decomposers microbes.

The Y-axis of each graph represents the abundance (reported either in percentage or in number 1x) of these microbes, compared to uBiome database control. Each column represents mean value of each treatment group (placebo, tulsi, rooibos and oolong). Along with the treatment types the X-axis in the bracket also contain number of participants analyzed for each group. The intensity of the color in the heatmap indicates the abundance of the respective microbiome.

Fig. S6. Herbal teas effects on (A) lipid metabolizing bacteria, and (B) TMA (metabolites of choline phospholipid [65]) producing bacteria.

The Y-axis of each graph represents the abundance (reported either in percentage or in number 1x) of these microbes, compared to uBiome database control. Each column represents mean value of each treatment group (placebo, tulsi, rooibos and oolong). Along with the treatment types the X-axis in the bracket also contain number of participants analyzed for each group. The intensity of the color in the heatmap indicates the abundance of the respective microbiome.
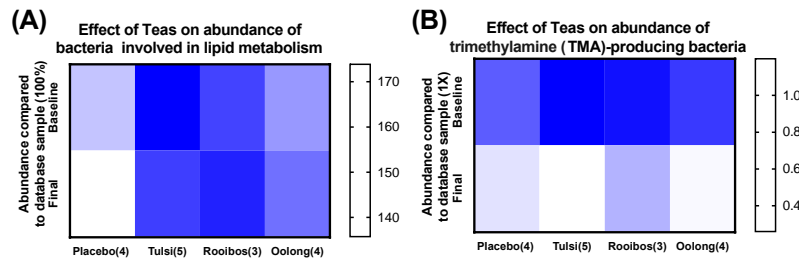

Fig. S7. Herbal teas effects on (A) vitamin-K producing bacteria, and (B) vitamin-B9 producing bacteria.

The Y-axis of each graph represents the abundance, reported in percentage of these microbes, compared to uBiome database control. Each column represents mean value of each treatment group (placebo, tulsi, rooibos and oolong). Along with the treatment types the X-axis in the bracket also contain number of participants analyzed for each group. The intensity of the color in the heatmap indicates the abundance of the respective microbiome.
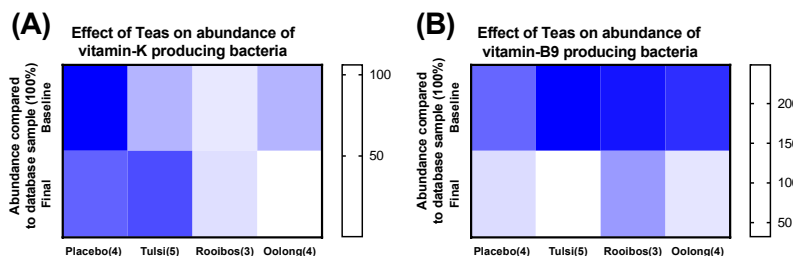

Fig. S8. Herbal teas effects on (A) lactobacillus, (B) Bifidobacterium, and (C) artificial sweeteners associated microbes.

The Y-axis of each graph represents the abundance reported in number 1x of these microbes, compared to uBiome database control. Each column represents mean value of each treatment group (placebo, tulsi, rooibos and oolong). Along with the treatment types the X-axis in the bracket also contain number of participants analyzed for each group. The intensity of the color in the heatmap indicates the abundance of the respective microbiome.
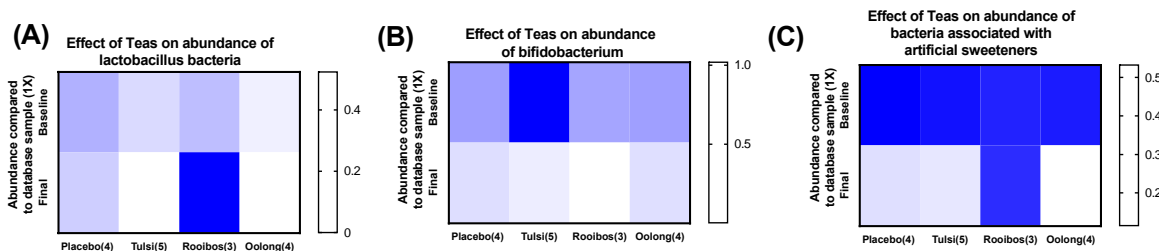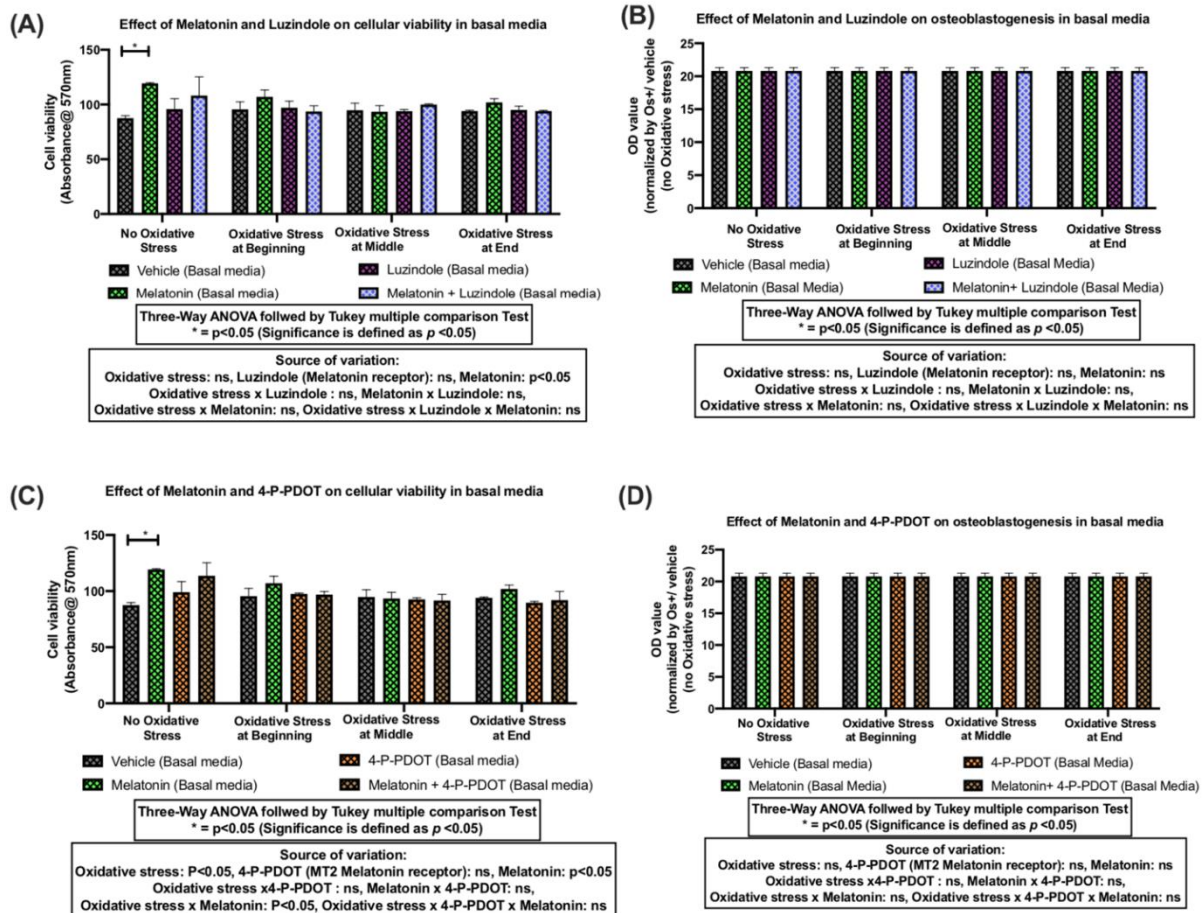

Fig. S9. Effect of melatonin on hMSCs in growth (basal) medium condition

hMSCs in basal media were exposed to melatonin in presence or absence of luzindole or 4-P-PDOT for 21 days and under normal and oxidative stress condition. Treatment effects on (A) cellular viability by MTT assay in presence of luzindole, (B) osteoblast differentiation via alizarin red staining in presence of luzindole (C) cellular viability by MTT assay in presence of 4-P-PDOT, (D) osteoblast differentiation via alizarin red staining in presence of 4-P-PDOT were assessed. Each bar represents the mean ($\pm$ SEM) absorbance of MTT at 570 nm or related OD of alizarin red staining for respective group performed in triplicate. MTT assay and ALZ staining values from all groups were normalized by vehicle treated group at no oxidative stress (Os+/Veh_No oxidative stress) for respective assay. Data were analyzed using Three-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (n=3 per group).

Table S1. Herbal teas effects on different groups and subgroups of gut microbiome from stool sample.

Group	Subgroup	Coriander (4)	Tulsi (5)	Rooibos (3)	Oolong (4)
Microbiome metabolizing Carbohydrate or metabolites (**Figure S5**)	Carbohydrate metabolizing	↑	↓	↑	↑
	lactose metabolizing	↓	↓	↓	↓
	Butyrate producing	↓	↓	↓	↓
	propionate producing	↓	↓	↓	↓
	Acetaldehyde accumulator	↓	↓	↓	↓
	Acetaldehyde decomposer	↓	↓	↓	↓
Microbiome metabolizing Amino acids or metabolites (**Figure 8**)	Amino acid metabolizing	↓	↓	↑	↑
	Polyamine producing	↓	↓	↓	↓
	GABA producing	↓	↓	↑	↑
	Serotonin producing	↑	↓	↓*	↑
Lipid metabolizing (**Figure S6**)	Lipid metabolizing	↓	↓	↑	↑
	TMA producing	↓	↓	↓	↓
Microbiome associated with micronutrients (**Figure S7**)	Vitamin-K producing	↓	↑	↑	↓
	Vitamin-B9 producing	↓	↓	↓	↓
Other Microbes (**Figure S8**)	Lactobacillus	↓	↓	↑	↓
	Bifidobacterium	↓	↓	↓	↓
	Related to artificial sweeteners	↓	↓	↓	↓

Summary table showing the changes in the abundance of microbiome compared to baseline. Along with the type of teas, in bracket number of samples analyzed were presented. *p < 0.05 vs. placebo, and red color arrow indicates direction of change different than placebo.

Table S2. Correlation co-efficient values between different parameters.

Parameters		correlation co-efficient (r)	Confidence Interval (95% CI)	Significance
P1NP	CTX	0.134	-0.224 to 0.462	p>0.5 (p=0.46)
P1NP	CRP	0.016	-0.334 to 0.363	p>0.5 (p=0.92)
CTX	CRP	-0.045	-0.388 to 0.308	p>0.5 (p=0.80)
P1NP/CTX	CRP	-0.003	-0.350 to 0.340	p>0.5 (p=0.98)
CTX	Melatonin	0.018	-0.332 to 0.364	p>0.5 (p=0.92)
P1NP	Melatonin	-0.087	-0.423 to 0.269	p>0.5 (p=0.63)
P1NP/CTX	Melatonin	-0.100	-0.433 to 0.257	p>0.5 (p=0.58)
Cortisol ratio	P1NP/CTX	0.083	-0.272 to 0.420	p>0.5 (p=0.64)
Cortisol ratio	PSS	-0.054	-0.395 to 0.300	p>0.5 (p=0.76)
Cortisol ratio	CES-D	-0.186	-0.502 to 0.173	p>0.5 (p=0.30)
Cortisol ratio	STAI-State anxiety	0.056	-0.298 to 0.397	p>0.5 (p=0.75)
Cortisol ratio	STAI-Trait anxiety	-0.236	-0.540 to 0.122	p>0.5 (p=0.19)
Cortisol ratio	QUALIOST- Emotional	0.010	-0339 to 0.357	p>0.5 (p=0.95)
Melatonin	QUALIOST- Physical	-0.071	-0.410 to 0.284	p>0.5 (p=0.69)
Melatonin	QUALIOST- Osteopenia	-0.041	-0.384 to 0.311	p>0.5 (p=0.82)
P1NP/CTX	QUALIOST- Osteopenia	0.181	-0.178 to 0.498	p>0.5 (p=0.31)