HHS Public Access

Author manuscript

Clin Pharmacol Transl Med. Author manuscript; available in PMC 2020 March 10.

Published in final edited form as:

Clin Pharmacol Transl Med. 2019 ; 3(2): 152–159.

Effects of C-Terminal Domain of the Heavy Chain of Tetanus Toxin on Gut Microbiota in a Rat Model of Depression

Bruk Getachew,

Yousef Tizabi

Department of Pharmacology, Howard University College of Medicine, Washington, DC, USA.

Abstract

Background/Aims: It is now well established that imbalance or dysbiosis in the gut microbiota (GM) plays a significant role in neuropsychiatric/neurodegenerative disorders. Recently it has been reported that the C-terminal domain of the heavy chain of tetanus toxin (Hc-TeTx) may not only act as a neuroprotectant but may also exhibit antidepressant effects in Wistar-Kyoto (WKY) rats, a putative animal model of treatment-resistant depression. The aim of this study was to determine whether Hc-TeTx may also interact with GM implicated in mood regulation in these rats.

Methods: Adult male WKY rats (5/group) were injected intramuscularly (IM) with 60 μg/kg Hc-TeTx or saline. Twenty-four hours after the injection, the animals were sacrificed, intestinal stools were collected and stored at −80°C. DNA was extracted from the samples for 16S rRNA gene-based microbiota analysis using 16S Metagenomics application.

Results: Abundance of several bacteria at different taxonomic levels were distinguished between Hc-TeTx group and the control. At species–level, 11 operational taxonomic units (OTUs), particularly *Bifidobacterium cholerium*, a bacterium with a strong ability to degrade resistant starch, were enriched (69 fold) in the Hc-TeTx group. In addition, 5 species of probiotic *Lactobacillus*, two butyrate-forming species S*arcina, Butyrivibro proteovlasticus* a*nd Roseburia faecis*, were enhanced by a minimum of 2-fold in Hc-TeTx group. In contrast, 24 species including five species of pathogenic *Provettela* (5–14 fold), two mucin-degrading *Akkermansia muciniphila and Mucispirillum schaedleri*, and four species of pathogenic *Ruminoccus* were reduced by a minimum of 2-fold by Hc-TeTx treatment.

Conclusion: Hc-TeTx enhanced probiotic species and suppressed the opportunistic pathogens. Since overall effect of Hc-TeTx appears to be promoting GM associated with mood enhancement (e.g. *Bifidobacterium, Butyrivibro, and Lactobacillus*) and suppressing GM associated with mood dysregulation (e.g. *Mucispirillum, Provettela, and Ruminoccus*) a novel mechanism of beneficial effects of Hc-TeTx may involve normalization of dysbiosis.

*Address for Correspondence: Dr. Yousef Tizabi, Ph.D., Department of Pharmacology, Howard University College of Medicine, 520 W Street NW, Washington DC 20059. USA, Tel:202-806-9719; Fax: 202-806-4453; ytizabi@howard.edu.

Conflict of Interest

The authors state that they have no conflict of interest.

Availability of supporting data

The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

Introduction

Major depressive disorder (MDD), a serious mental health problem with heavy economic burden worldwide [1], ranks as one of the leading causes of disability [2]. A number of drugs such as, tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), norepinephrine reuptake inhibitors (NRIs), and selective serotonin reuptake inhibitors (SSRIs), developed over the past 6 decades have offered significant relief to at least some of the patients [3, 4]. These medications, however, have limited efficacy, delayed onset and various undesirable side effects [4]. More recently, (S)-ketamine (esketamine), a glutamatergic NMDA receptor antagonist was approved by FDA for use in treatment-resistant depression (FDA News Release, March 5, 2019). However, esketamine can cause drowsiness and must be administered in a healthcare setting. Therefore, more efficacious drugs with less side effects are urgently needed, Understanding the complex neurobiological substrate of mood disorders is critical in development of novel antidepressants.

The high co-morbidity of gastrointestinal (GI) disorders, such as colitis or inflammatory bowel disease (IBD) and depression, suggests that imbalance in gut microbiome (GM) or dysbiosis may play an important role in both these conditions [5–9]. Further support for this hypothesis comes from studies that show pharmacotherapy of mood disorders may actually be beneficial to GI disorders through GM manipulation [10, 11], and that some of the drugs that are used in ulcerative colitis or IBD can have mood elevating effects [12, 13]. Thus, interaction of antidepressants with GM may not only indicate another distinct mechanism of action for these drugs but may also offer a novel target for development of more effective antidepressants.

The carboxyl-terminal domain of the heavy chain of tetanus toxin (Hc-TeTx) was recently shown to have an antidepressant effect in Wistar-Kyoto rats, a putative animal model of depression [14]. In this study we sought to determine whether Hc-TeTx may also affect the microbiota in the gut that has been implicated in mood regulation.

Methods

Animals

Age matched, approximately 3 months old adult male Wistar Kyoto (WKY) rats (Envigo, USA) were housed 2–3 per cage in standard polypropylene shoebox cages (42 × 20.5 × 20 cm) on hardwood chip bedding (alpha-dry) in a designated room. Throughout the experiment, animals had access to food (Harlan Tek Lab) and water ad libitum. The room was maintained at 24–26 °C at 51–66% relative humidity, on a 12-h light/dark cycle (lights on at 7 am).

In order to acclimate the animals to the housing conditions, animals arrived at least one week prior to initiation of any experiment. During this period, they were gentled once daily in order to minimize any stress effects that might result from routine handling. All experiments were carried out in accordance with NIH guidelines, as approved by the Institutional Animal Care and Use Committee of the Howard University.

Drugs

Hc-TeTx fragment, generously donated by Dr. José Aguilera, was synthesized as described in detail previously [15, 16]. The Hc-TeTx solution was prepared by dissolving 1 mg of lyophilized Hc-TeTx in 1 mL of isotonic saline solution, followed by serial dilutions to obtain a final concentration of 60 μg/ 100 μL. The volume of injection was 400 μL/kg. Hence each animal received approximately 100 μL of saline or the drug. This doss was chosen to match the dose that had resulted in maximal antidepressant effect in the same animals [14].

Experimental design

Following one week of acclimation, the animals were randomly divided into two groups, control and experimental (n=5 each) and were housed in separate cages. Animals belonging to the same group were also randomly selected and housed together (2–3 animals/cage). This housing method assured that the animals in both groups were exposed to identical environment and that there would not be any cross contamination between the treated vs the control group.

Control group was injected with saline, whereas the experimental group received Hc-TeTx (60 μg/kg) into the right gastrocnemius muscle.

Sample collection

Approximately 24 h after the Hc-TeTx or saline injection, the animals were sacrificed by decapitation, alternating between the groups. Colons containing stools were collected, quick-frozen on dry ice and stored at −80°C. This method of rapid-freezing is considered best-practice for preserving stool DNA samples [17].

Stool DNA extraction

Total DNA was isolated from stool samples using Norgen's Stool DNA Isolation Kit and the Precellys Dual-24 Homogenizer (Bertin Technologies). Purification was based on spin column chromatography using resin as the separation matrix. Briefly, 200 mg stool samples were bead-homogenized after adding 1 mL of Lysis Buffer L. One hundred μL of lysis additive was added and vortexed, followed by centrifugation at 20,000 × g for 5 min. The clear supernatant was transferred (600 μL) to a DNAase-free microcentrifuge tube. Next, the samples were centrifuged and 100 μL of Binding Buffer I was added to the clean supernatant and incubated on ice for 10 minutes. Equal amounts of 70% ethanol were then added to the clean supernatant from Binding Buffer I lysate after centrifugation. The protocol was then followed for complete DNA isolation. The purified DNA was quantified and analyzed for purity using the NanoDrop™ 2000 Spectro-photometer (NanoDrop Technologies, Wilmington, DE). Twenty μL of purified DNA was then quick-frozen on dry ice and shipped to Norgen Biotek (Thorold, ON, Canada) for 16S rRNA gene analysis.

16S rRNA gene sequencing and Analysis

Briefly, the V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified from 12.5 ng of stool DNA. The amplicons were then cleaned, sequenced according

to the Illumina MiSeq 16S Metagenomic Sequencing Library Preparation protocol (http://support.illumina.com/downloads/16s_metagenom”ic_sequencing_library_preparation) [18]. The final library was paired-end sequenced at 2 × 300 bp using a MiSeq Reagent Kit v3 on the Illumina MiSeq platform. For bioinformatic analysis, the sequencing data was analyzed using the Illumina 16S metagenomics app (Illumina 16S Metagenomics Pippeline (v1.0.1) (https://basespace.illumina.com/apps/593593/16SMetagenomics/perferredversion) [19], which performs taxonomic classification of 16S rRNA targeted amplicons using an Illumina-curated version of the GeenGenes taxonomic database. The app provides interactive visualization and raw classification output for per-sample and aggregate analyses. Classification was performed using the Illumina 16 S Metagenomics workflow, which is also available in the MiSeq Reporter software. The algorithm uses a high-performance implementation of the Ribosomal Database Project (RDP) Classifier described in Wang Q et al., 2007 [20].

Statistical analysis

Since comparison was performed between two groups with equal variance (Hc-TeTx- and saline-treated animals), Student T-test was applied for detecting significant differences in specific measured parameters. The cut-off for statistical significance was $p<0.05$, two-tailed.

Results

Diversity and Richness

Figure 1 depicts effects of Hc-TeTx (60 μg/kg i.m.) on gut bacterial species diversity (A) and species richness (B). A total of 1,138 different bacterial species were identified in both saline and Hc-TeTx groups. Overall, there was no significant difference in either diversity [Figure. 1a) as estimated by the Shannon Diversity Index (SDI) (saline control vs. Hc-TeTx; 2.59 vs. 2.55, P=0.61. or species richness [Figure. 1b] as measured by mean species number. Although a total of 1,138 species were identified, only less than 650 were considered qualified (i.e., made the cut off at 0.01% abundance, saline control: 607; Hc-TeTx: 596, P=0.55).

Taxa-level distribution

There were a total number of 29 Phyla, 56 classes, 106 orders, 234 families, 600 genera and 1,138 species identified in the two groups. There were no differences between the saline and Hc-TeTx group in percent reads, i.e., percentage of identified sequences belonging to each taxon [Figure 2].

Phylum-Level Effects

Hc-TeTx significantly reduced abundances of D*eferribacteres, Bacteroidetes, Protebacteria, Tenericutes and Spirochaetes* [Figure 3]. Overall there were 29 different phyla identified in the two groups. *Deferribacteres, Bacteroidetes, Protebacteria, Tenericutes and Spirochaetes* are all low-abundance phyla accounting for less than 2% of the total phyla reads. Hc-TeTx selectively reduced *Deferribacteres, Bacteroidetes, Protebacteria, Tenericutes and Spirochaetes* by minimum of 2-fold compared to saline control group.

Class-Level Effects

Hc-TeTx significantly reduced the levels of three classes, Deferribacteres, Alphaproteobacteria, Spirochaetes compared to saline group by minimum of 2-fold each and enriched Verrucomicrobiae compared to saline group by 4-fold. There was a total of 56 different classes identified between the two groups.

Order-Level Effects

Hc-TeTx significantly increased Turicibacterales, and Bifidobacteriales by 2-and 4-fold respectively, and decreased Burkholderiales, Desulfuromonadales, Rhodospirillales, Deferribacterales, Anaeroplasmatales and Rhizobiales by minimum of 2-fold, compared to saline group. There was a total of 106 different orders identified between the two groups [Figure 5].

Family-Level Effects

Hc-TeTx significantly enriched abundance of genera Turicibacteraceae, Microbacteraceae, Bifidobacteriaceae, Streptomycetaceae, and Verrucomicrobiaceae by minimum of 2-fold. Conversely, Hc-TeTx reduced abundance of Prevotellaceae, Rhodospirillaceae, Deferrribacteraceae, Dehalobacteriaceae, and Heliobacteraceae by minimum of 2-fold compared to saline group [Figure 6]. There was a total of 234 different families. It should be noted that for analysis at the family level, the samples were pooled and hence overall there were two groups to be compared (control vs treated). Since a statistical analysis could not be performed in such cases, we used a conservative cut-off point of a minimum of 2-fold difference between the groups, which could imply important changes.

Genus-Level Effects

Hc-TeTx significantly enriched abundances of 14 genera particularly, Anaeroplasma, Bifidobacterium, Butyrivibrio, Kitasatospora, Lactobacillus, Olivibacter, Roseburia, Sarcina, and Turicibacter by a minimum of 2-fold. Conversely, Hc-TeTx decreased levels of Actinomyces, Anaeroplasma, Coprococcus, Dehalobacterium, Erysipelothrix, Heliorestis, Mesoplasma, Mucispirillum, Novispirillum, Oscillospira, Parapedobacter, Peptoniphilus, Provottella, Rhodospirillum, Ruminococcoccus, and Sutterella by a minimum of 2-fold [Figure 7]. All comparisons are with respect to saline group. There was a total of 611 different genera. It should be noted that for analysis at the genus level, the samples were pooled and hence overall there were two groups to be compared (control vs treated). Since a statistical analysis could not be performed in such cases, we used a conservative cutoff point of a minimum of 2-fold difference between the groups, which could imply important changes.

Species-Level Effects

Hc-TeTx significantly enriched abundances of 11 species particularly, 69-fold increase in Bifidobacterium cholerinum, and more than 2-fold increase in five species of Lactobacillus, Butyrivibrio proteoclasticus, Rosburia facecien. On the other hand, levels of 24 species including: three bacteroides, two Balutia, three Clostridium, three Desulfovibrio, two Erythbacterium, five Prevotella and four Ruminococcus were reduced by minimum of

2-fold, compared to saline control group [Figure 8]. There were a total of 1138 different species identified. It should be noted that for analysis at, the species level, the samples were pooled and hence overall there were two groups to be compared (control vs treated). Since a statistical analysis could not be performed in such cases, we used a conservative cutoff point of a minimum of 2-fold difference between the groups, which could imply important changes.

Discussion

The results of this study indicate that a single injection of Hc-TeTx in WKY rats promoted certain gut bacteria associated with mood enhancement (e.g. Akkermansia, Bifidobacterium and Lacto-bacillus) and suppressed those associated with inflammation (e.g. Ruminoccus, Mucispirillum and Provettela). Since inflammation can be a major contributor to mood dysregulation and pathogenesis of depression [21–25], it might be suggested that at least part of Hc-TeTx antidepressant effects are mediated through its interaction with the GM. Moreover, the behavioral disparity including depressive-like characteristics, stress and ulcer susceptibility in WKY rats might also be influenced by the dysbiosis in GM of this strain.

GM dysbiosis has also been implicated in neurodegenerative diseases, particularly Parkinson's disease (PD) [26]. Interestingly, a high co-morbid occurrence of depression and PD has also been noted [22]. Since, neuroprotective effects of Hc-TeTx in various in-vitro and in-vivo models of PD have been documented [16, 27–29], it may be suggested that this compound could have specific utility in PD-depression co-morbidity and that at least some of its actions are mediated via interaction with the microbiota in the gut.

A common denominator in both depression and PD may be an exaggerated inflammatory response, particularly in terms of microglial activation in the brain [30–33]. WKY rats as animal model of treatment-resistant depression are also susceptible to stress-induced ulceration, which has been attributed to release of pro-inflammatory cytokines [34–36]. Moreover, these animals show significant dysbiosis [14]. Since Hc-TeTx has considerable anti-inflammatory properties, including suppression of microglia activation in the brain [16] as well capability of normalizing dysbiosis as seen in this study, it may be advocated as a suitable candidate for depression and/or PD, and also in chronic intestinal inflammatory disorders.

As mentioned earlier, (S)-ketamine was recently approved by FDA for use in treatment-resistant depression. Interestingly, ketamine was also shown to interact with many of the microbiota in the gut that were also affected by Hc-TeTx [37]. In this regard, it would be of significant interest to determine the effect of classical as well as potential novel antidepressants on GM. Moreover, since antidepressants are likely to have neuroprotectant effects and neuroprotectants are likely to have antidepressant effects [38], their interaction with GM may suggest novel therapeutic interventions involving pre- or pro-biotics. A recent report indicating potential utility of butyrate forming prebiotics in PD [26], gives further credence to this hypothesis. Indeed, "butyrogenic" bacteria may influence inflammatory conditions and hence be of benefit in neurological and/or neuropsychiatric diseases [39–42]. Since Hc-TeTx significantly increases the levels of butyrogenic bacteria (e.g. *Lactobaccillus,*

Bifidobacterium), it may be suggested that at least some of its beneficial effects may be mediated via this mechanism.

Curiously, the Westernized diet, implicated in metabolic syndrome, is linked to lower levels of *Lactobacillus* and *Sarcina*, and higher levels of *Ruminococcus* [43, 44], all of which were affected by Hc-TeTx. Thus, Hc-TeTx increased the levels of beneficial "probiotics" *Lactobacillus and Sarcina*, and decreased levels of the "opportunistic" *Ruminococcus*. Hence, it may be of benefit in metabolic syndrome as well. Moreover, some of the effects of Hc-TeTx on GM are similar to recently reported GM effects of Tianasi Liquid, a Chinese herbal medicine, advocated for its metabolomic effects [45]. It is important, however, to note that *Lactobacillus* genus members are unable to synthesize amino acids and/or purines and thus rely on nutrient rich environments and other bacteria for supply of essential building blocks [40, 42, 46, 47]. Therefore, the presence of other probiotic bacteria such as *Bifidobacterium and Lachnospira* may be required for *Lactobaccillus* to produce therapeutic benefits [39–41, 46, 47].

Some species of *Mucispirillum* may cause intestinal inflammation by causing a "leaky" gut, where there is an increase in gut permeability due to degradation of mucin (colonic mucus layer), which is crucial in maintaining the physical barrier that separates trillions of gut bacteria from the host [48–50]. Leaky gut is also a key contributor to the co-morbidity of depression and intestinal disorders [51–53]. In addition, *Mucispirillum* is positively associated with increases in plasma levels of lipopolysaccharide (LPS) and is considered colitogenic. Indeed, because of this property, it is used as a microbial marker of active colitis [51–53]. On the other hand, low levels of *Sarcina* have been implicated in inflammatory processes of relevance to depression [21, 54, 55]. Thus, the dramatic reduction (over 9-fold) of *Mucispirillum*, and over a 2-fold increase in *Sarcina* by Hc-TeTx, strengthens its potential usefulness in leaky gut conditions as well.

In summary, our findings indicate that a single administration of Hc-TeTx results in significant increases in the levels of probiotic bacteria (e.g. *Lactobacillus, Bifidobacterium and Butyrivibrio*), and significant decreases in opportunistic pathogens (e.g. *Provettela and Mucispirallum*) in WKY rats, a putative animal model of depression. Since these microorganisms have been implicated in mood regulation and inflammatory diseases, the utility of He-TeTx in these conditions as well as in "leaky" gut warrants further investigation.

Acknowledgment

Supported in part by R03AA022479 from NIAAA

References

1. Kassebaum NJ, Barber RM, Bhutta ZA, Dandona L, Gething PW, Hay SI, et al. Global, regional, and national levels of maternal mortality, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016; 388:1775–1812. [PubMed: 27733286]
2. WHO: World Health Organization. 2014, June 1. Estimates for 2000–2012. Retrieved October 19, 2015.

3. Schildkraut JJ. The catecholamine hypothesis of affective disorders: A review of supporting evidence. Am J Psychiatry. 1965; 122:509–522. [PubMed: 5319766]
4. Elizabeth P, Tracy DK: The drugs don't work? antidepressants and the current and future pharmacological management of depression. Ther Adv Psychopharmacol. 2012; 2:179–188. [PubMed: 23983973]
5. De Palma G, Collins SM, Bercik P, Verdu EF. The microbiota-gut-brain axis in gastrointestinal disorders: stressed bugs, stressed brain or both? J Physiol. 2014; 592:2989–2897. [PubMed: 24756641]
6. Kelly JR, Borre Y, O'Brien C, Patterson E, El Aidy S, Deane J, et al. Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. J Psychiatr Res. 2016; 82:109–118. [PubMed: 27491067]
7. Clapp M, Aurora N, Herrera L, Bhatia M, Wilen E, Wakefield S. Gut microbiota's effect on mental health: the gut-brain axis. Clin Pract. 2017; 7:987. [PubMed: 29071061]
8. Martin CR, Mayer EA. Gut-brain axis and behavior. Nestle Nutr Inst Workshop Ser. 2017; 88:45–53. [PubMed: 28346923]
9. Kappelmann N, Lewis G, Dantzer R, Jones PB, Khandaker GM. Antidepressant activity of anti-cytokine treatment: a systematic review and meta-analysis of clinical trials of chronic inflammatory conditions. Mol Psychiatry. 2018; 23:335–343. [PubMed: 27752078]
10. Mikocka-Walus AA. Treatment of psychological co-morbidities in common gastrointestinal and hepatologic disorders. World J Gastrointest Pharmacol Ther. 2010; 1:64–71. [PubMed: 21577298]
11. Sampson TR, Mazmanian SK. Control of brain development, function, and behavior by the microbiome. Cell Host Microbe. 2015; 17:565–576. [PubMed: 25974299]
12. Foxx-Orenstein AE. New and emerging therapies for the treatment of irritable bowel syndrome: an update for gastroenterologists. Therap Adv Gastroenterol. 2016; 9:354–375.
13. Keerthy D, Youk A, Srinath AI, Malas N, Bujoreanu S, Bousvaros A, et al. Effect of psychotherapy on health care utilization in children with inflammatory bowel disease and depression. J Pediatr Gastroenterol Nutr. 2016; 63:658–664. [PubMed: 27035372]
14. Getachew B, Mendieta L, Csoka AB, Aguilera J, Tizabi Y. Antidepressant effects of C-Terminal domain of the heavy chain of tetanus toxin in a rat model of depression. Behav Brain Res. 2019; 370:111968. [PubMed: 31125623]
15. Herrando-Grabulosa M, Casas C, Aguilera J. The C-terminal domain of tetanus toxin protects motoneurons against acute excitotoxic damage on spinal cord organotypic cultures. J Neurochem. 2013; 124:36–44. [PubMed: 23106494]
16. Patricio F, Parra I, Martínez I, Pérez-Severiano F, Montes S, Aguilera J, Limón ID, Tizabi Y, Mendieta L. Effectiveness of fragment C domain of tetanus toxin and pramipexole in an animal model of Parkinson's disease. Neurotox Res. 2019; 35:699–710. [PubMed: 30607904]
17. Mathay C, Hamot G, Henry E, Georges L, Bellora C, Lebrun L, et al. Method optimization for fecal sample collection and cecal DNA extraction. Biopreserv Biobank. 2015; 13:79–93. [PubMed: 25880472]
18. Illumina MiSeq 16S metagenomic sequencing library preparation protocol. Accessed 25 Aug 2017.
19. Illumina 16S metagenomics pipeline (v1.0.1). Accessed 20 Aug 2017.
20. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007; 73:5261–5267. [PubMed: 17586664]
21. Getachew B, Aubee JI, Schottenfeld RS, Csoka AB, Thompson KM, Tizabi Y. Ketamine interactions with gut-microbiota in rats: relevance to its antidepressant and anti-inflammatory properties. BMC Microbiol. 2018; 18:222. [PubMed: 30579332]
22. Tizabi Y, Getachew B, Csoka AB, Manaye KF, Copeland RL. Novel targets for parkinsonism-depression comorbidity. Prog Mol Biol Trans Med. 2019; 167:1–24.
23. Guo Y, Xie JP, Deng K, Li X, Yuan Y, Xuan Q, Xie J, He XM, Wang Q, Li JJ, Luo HR. Prophylactic effects of Bifidobacterium adolescentis on anxiety and depression-like phenotypes after chronic stress: A role of the gut microbiota-inflammation axis. Front Behav Neurosci. 2019; 13:126. [PubMed: 31275120]

24. Hills RD Jr, Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. Gut microbiome: profound implications for diet and disease. Nutrients. 2019; 11.
25. Cheng LH, Liu YW, Wu CC, Wang S, Tsai YC. Psychobiotics in mental health, neurodegenerative and neurodevelopmental disorders. J Food Drug Anal. 2019; 27:632–648. [PubMed: 31324280]
26. Cantu-Jungles TM, Rasmussen HE, Hamaker BR. Potential of Prebiotic Butyrogenic Fibers in Parkinson's Disease. Front Neurol. 2019; 10:663. [PubMed: 31281287]
27. Sánchez-González A, Mendieta L, Palafox V, Candalija A, Luna F, Aguilera J, Limón ID. The restorative effect of intramuscular injection of tetanus toxin C-fragment in hemiparkinsonian rats. Neurosci Res. 2014; 84:1–9. [PubMed: 24815514]
28. Mendieta L, Granado N, Aguilera J, Tizabi Y, Moratalla R. Fragment C Domain of Tetanus Toxin Mitigates Methamphetamine Neurotoxicity and Its Motor Consequences in Mice. Int J Neuropsychopharmacol. 2016; 19.
29. Moreno-Galarza N, Mendieta L, Palafox-Sánchez V, Herrando-Grabulosa M, Gil C, Limón DI, Aguilera J. Peripheral Administration of Tetanus Toxin Hc Fragment Prevents MPP+ Toxicity In Vivo. Neurotox Res. 2018; 34:47–61. [PubMed: 29460114]
30. Gao HM, Hong JS. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends Immunol. 2008; 29:357–365. [PubMed: 18599350]
31. Kim YJ, Park HJ, Lee G, Bang OY, Ahn YH, Joe E, et al. Neuroprotective effects of human mesenchymal stem cells on dopaminergic neurons through anti-inflammatory action. Glia. 2009; 57:13–23. [PubMed: 18661552]
32. Hurley LL, Tizabi Y. Neuroinflammation, neurodegeneration, and depression. Neurotox Res. 2013; 23:131–134. [PubMed: 22895696]
33. Joers V, Tansey MG, Mulas G, Carta AR. Microglial phenotypes in Parkinson's disease and animal models of the disease. Prog Neurobiol. 2017; 155:57–75. [PubMed: 27107797]
34. Paré WP. Stress ulcer susceptibility and depression in Wistar Kyoto (WKY) rats. Physiol Behav. 1989; 46:993–998. [PubMed: 2634265]
35. Bregonzio C, Artmando I, Ando H, Jezova M, Baiardi G, Saavedra JM. Anti-inflammatory effects of angiotensin II AT1 receptor antagonism prevent stress-induced gastric injury. Am J Physiol Gastrointest Liver Physiol. 2003; 285:G414–423. [PubMed: 12686508]
36. Bevivino G, Monteleone G. Advances in understanding the role of cytokines in inflammatory bowel disease. Expert Rev Gastroenterol Hepatol. 2018; 12: 907–915. [PubMed: 30024302]
37. Getachew B, Reyes RE, Davies DL, Tizabi Y. Moxidectin Effects on Gut Microbiota of Wistar-Kyoto Rats: Relevance to Depressive-Like Behavior. Clin Pharmacol Transl Med. 2019; 3:134–142. [PubMed: 31321385]
38. Tizabi Y Duality of Antidepressants and Neuroprotectants. Neurotox Res. 2016; 30:1–13. [PubMed: 26613895]
39. Davie JR. Inhibition of histone deacetylase activity by butyrate. J Nutr. 2003; 133:2485S–93S. [PubMed: 12840228]
40. Biddle AS, Black SJ, Blanchard JL. An in vitro model of the horse gut microbiome enables identification of lactate-utilizing bacteria that differentially respond to starch induction. PLoS One. 2013; 8:e77599. [PubMed: 24098591]
41. Donohoe DR, Curry KP, Bultman SJ. Microbial oncotarget: bacterial- produced butyrate, chemoprevention and Warburg effect. Oncotarget. 2013; 4:182–183. [PubMed: 23563701]
42. Stilling RM, van de Wouw M, Clarke G, Stanton C, Dinan TG, Cryan JF. The neuropharmacology of butyrate: the bread and butter of the microbiota- gut-brain axis? Neurochem Int. 2016; 99:110–132. [PubMed: 27346602]
43. Rossi G, Pengo G, Caldin M, Palumbo Piccionello A, Steiner JM, et al. Comparison of microbiological, histological, and immunomodulatory parameters in response to treatment with either combination therapy with prednisone and metronidazole or probiotic VSL#3 strains in dogs with idiopathic inflammatory bowel disease. PLoS One. 2014; 9:e94699. [PubMed: 24722235]
44. Martínez I, Stegen JC, Maldonado-Gómez MX, Eren AM, Siba PM, Greenhill AR, et al. The gut microbiota of rural Papua new Guineans: composition, diversity patterns, and ecological processes. Cell Rep. 2015; 11:527–538. [PubMed: 25892234]

45. Cheng D, Chang H, Ma S, Guo J, She G, Zhang F, Li L, Li X, Lu Y. Tiansi Liquid Modulates Gut Microbiota Composition and Tryptophan Kynurenine Metabolism in Rats with Hydrocortisone-Induced Depression. Molecules. 2018; 23. pii: E2832. [PubMed: 30384480]

46. Duncan SH, Louis P, Flint HJ. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl Environ Microbiol. 2004; 70:5810–5817. [PubMed: 15466518]

47. Yan W, Sun C, Yuan J, Yang N. Gut metagenomic analysis reveals prominent roles of lactobacillus and cecal microbiota in chicken feed efficiency. Sci Rep. 2017; 7:45308. [PubMed: 28349946]

48. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008; 9:46–56. [PubMed: 18073775]

49. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes. 2008; 57:1470–1481. [PubMed: 18305141]

50. Turnbaugh PJ, Gordon JI. The core gut microbiome, energy balance and obesity. J Physiol. 2009; 587:4153–4158. [PubMed: 19491241]

51. Goyal A, Yeh A, Bush BR, Firek BA, Siebold LM, Rogers MB, et al. Safety, clinical response, and microbiome findings following fecal microbiota transplant in children with inflammatory bowel disease. Inflamm Bowel Dis. 2018; 24:410–421. [PubMed: 29361092]

52. Laserna-Mendieta EJ, Clooney AG, Carretero-Gomez JF, Moran C, Sheehan D, Nolan JA, et al. Determinants of reduced genetic capacity for butyrate synthesis by the gut microbiome in Crohn's disease and ulcerative colitis. J Crohns Colitis. 2018; 12:204–216. [PubMed: 29373727]

53. Webb RC, Bakker DH, Koboziev I, Jones-Hall Y, Kottapalli RK, Ostanin D, et al. Differential susceptibility to T cell-induced colitis in mice: role of the intestinal microbiota. Inflamm Bowel Dis. 2018; 24:361–379. [PubMed: 29361089]

54. Ratuapli SK, Lam-Himlin DM, Heigh RI. Sarcina ventriculi of the stomach: a case report. World J Gastroenterol. 2013; 19:2282–2285. [PubMed: 23599657]

55. Im JY, Sokol S, Duhamel GE. Gastric dilatation associated with gastric colonization with sarcina-like bacteria in a cat with chronic enteritis. J Am Anim Hosp Assoc. 2017; 53:321–325. [PubMed: 28892423]

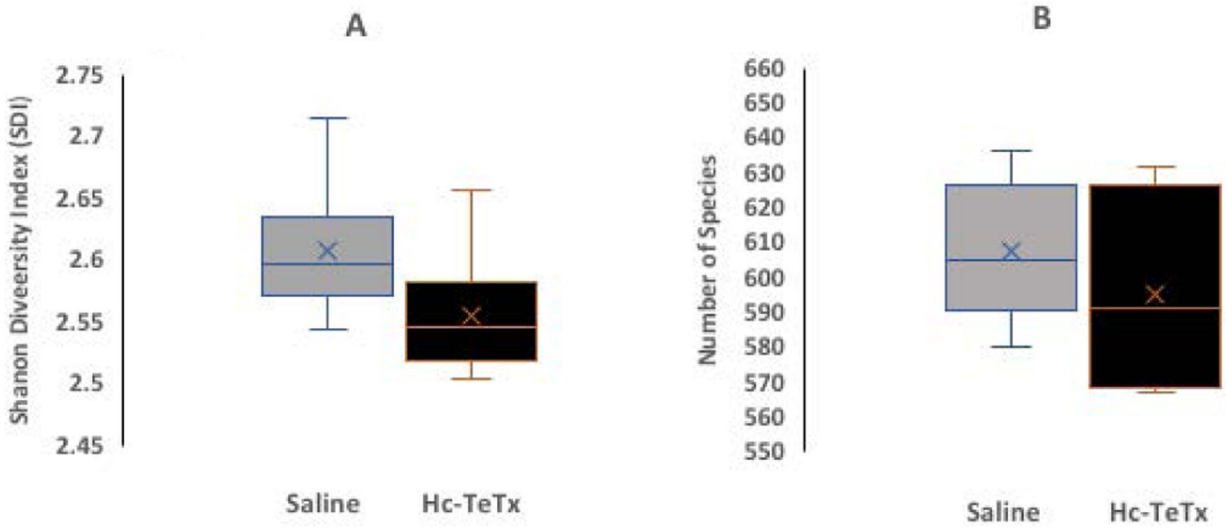

Figure 1:

Effects of Hc-TeTx (60 μg/kg i.m.) on gut bacterial species diversity (a) and species richness (b). Box-and-Whisker represent values of Shannon Diversity Index (n=5) for (a) and species number (b). Hc-TeTx did not have any significant effect on species diversity (P=0.61) or species richness (P=0.55). The 'x' designation in the middle of the bar represents mean value for each group, horizontal line across the bars indicate median values. The minimum and maximum quartile values are represented by a horizontal line in the bottom and top of the bars.

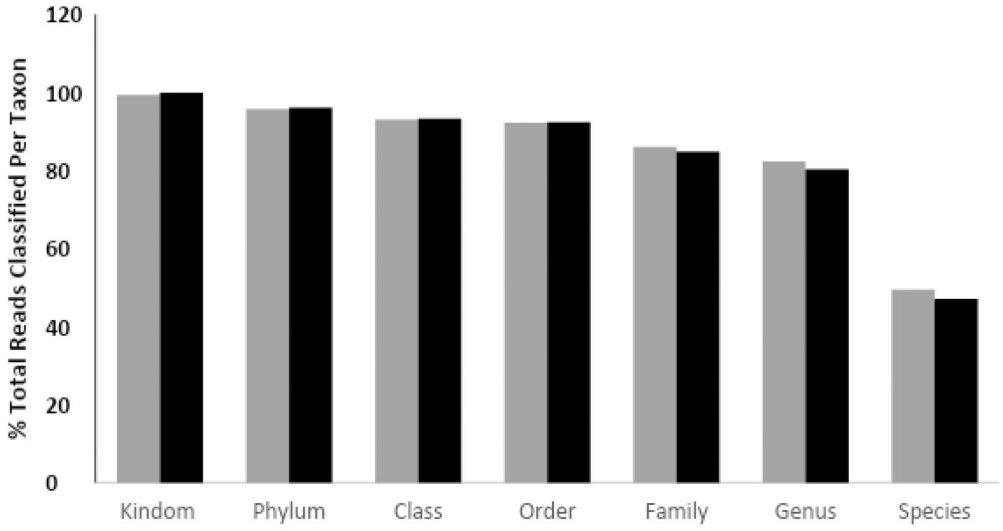

Figure 2:

Effects of Hc-TeTx (60 μg/kg i.m.) on gut bacterial distribution of taxa. Values are percent total reads/taxa/group (n=5). There were no significant differences in the % reads in taxon between saline and Hc-TeTx group. Note: the samples from each group were pooled and hence overall there were two groups to be compared (control vs treated).

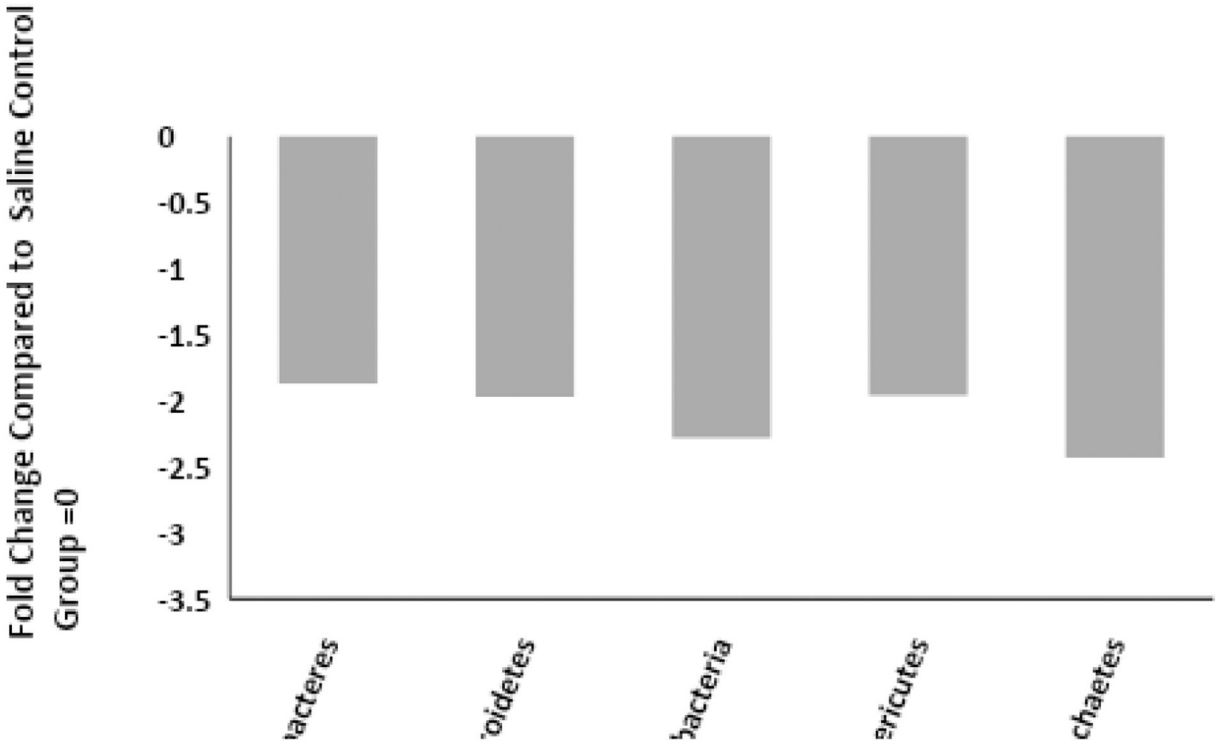

Figure 3:

Effects of Hc-TeTx (60 μg/kg i.m.) on fold change in abundance of gut bacterial phylum. Values are the fold change of mean compared to saline-control group (n=5). Hc-TeTx substantially decreased *Deferribacteres, Bacteroidetes, Protebacteria, Tenericutes and Spirochaetes* compared to saline group by minimum of 2-fold each. Note: the samples from each group were pooled and hence overall there were two groups to be compared (control vs treated).

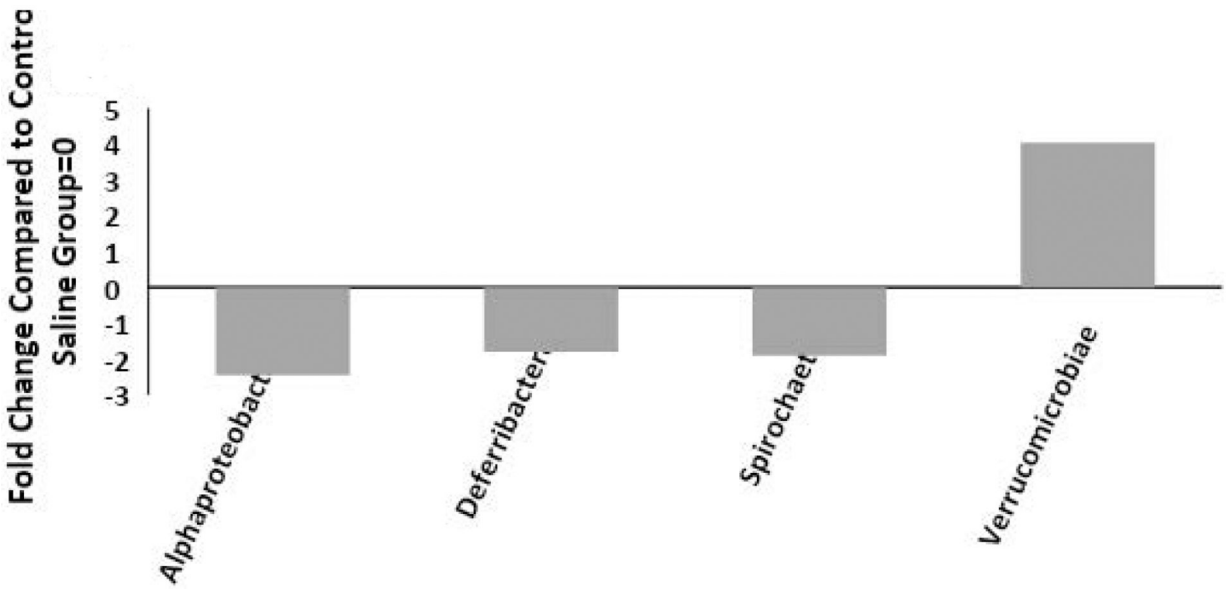

Figure 4:

Effects of Hc-TeTx (60 μg/kg i.m.) on fold change in abundance of gut bacterial class. Values are the fold change over saline group mean (n=5). Hc-TeTx significantly decreased *Deferribacteres, Alphaproteobacteria, Spirochaetes* compared to saline group by minimum of 2-fold respectively, and enriched Verrucomicrobiae compared to saline group. Note: the samples from each group were pooled and hence overall there were two groups to be compared (control vs treated).

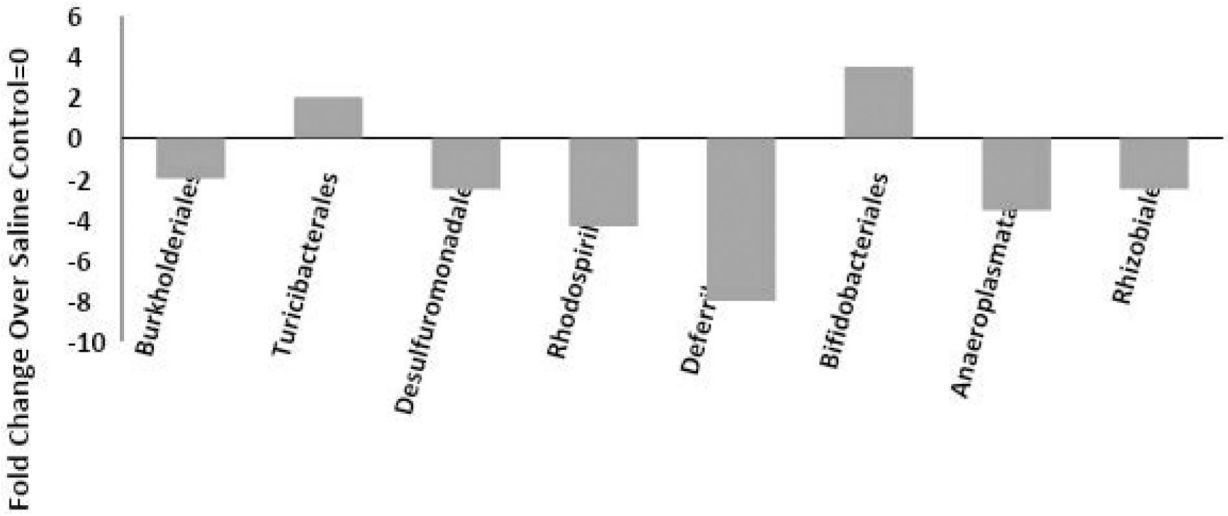

Figure 5:

Effects of Hc-TeTx (60 μg/kg i.m.) on fold change in abundance of gut bacterial order. Values are the fold change over saline control group mean (n=5). Hc-TeTx significantly increased Turicibacterales, and Bifidobacteriales by 2- and 4-fold respectively, and decreased Burkholderiales, Desulfuromonadales, Rhodospirillales, Deferribacterales, Anaeroplasmatales and Rhizobiales by minimum of 2-fold compared to saline group. There was a total of 106 different orders. Note: the samples from each group were pooled and hence overall there were two groups to be compared (control vs treated).

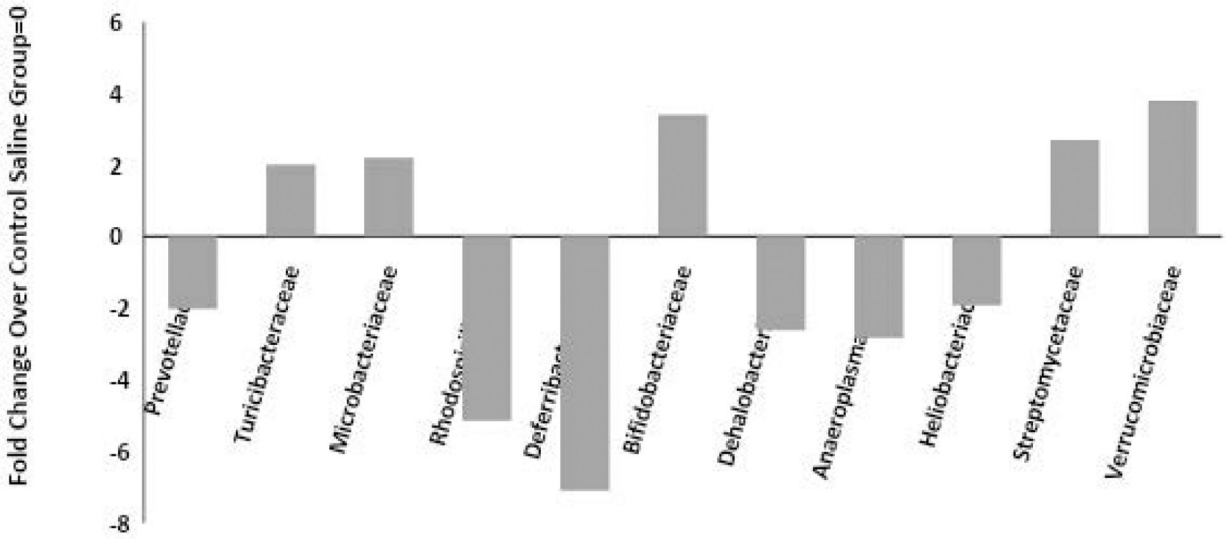

Figure 6:

Effects of Hc-TeTx (60 μg/kg i.m.) on fold change in abundance of gut bacterial family. Values are the fold change over saline group mean (n=5). Hc-TeTx significantly enriched abundance of genera Turicibacteraceae, Microbacteraceae, Bifidobacteriaceae, Streptomycetaceae, and Verrucomicrobiaceae by minimum of 2-fold. Conversely, Hc-TeTx reduced abundance of Prevotellaceae, Rhodospirillaceae, Deferrribacteraceae, Dehalobacteriaceae, and Heliobacteraceae by minimum of 2-fold compared to saline group. There was a total of 234 families.

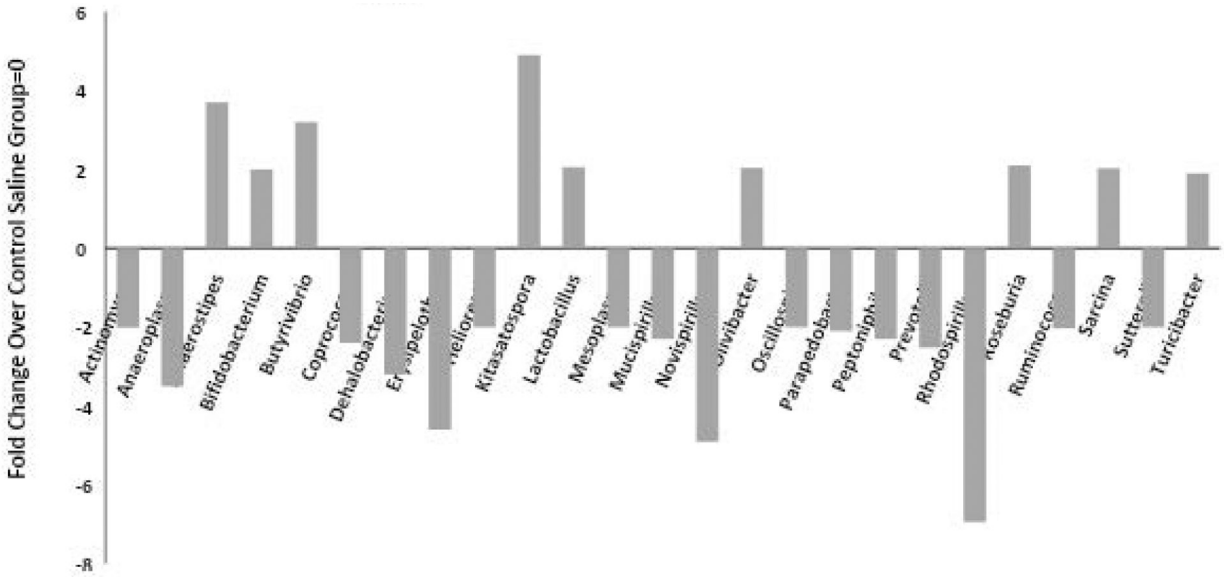

Figure 7:

Effects of Hc-TeTx (60 μg/kg i.m.) on fold change in abundance of gut bacteria genus. Values are the fold change over saline control group mean (n=5). Hc-TeTx significantly enriched abundance of 14 genera, particularly, Anaeroplasma, Bifidobacterium, Butyrivibrio, Kitasatospora, Lactobacillus, Olivibacter, Roseburia, Sarcina, and Turicibacter by minimum of 2-fold. Conversely, Hc-TeTx decreased levels of Actinomyces, Anaeroplasma, Coprococcus, Dehalobacterium, Erysipelothrix, Heliorestis, Mesoplasma, Mucispirillum, Novispirillum, Oscillospira, Parapedobacter, Peptoniphilus, Provottella, Rhodospirillum, Ruminococcoccus, and Sutterella by a minimum of 2-fold. All comparisons are with respect to saline group. There was a total of 611 different genera.

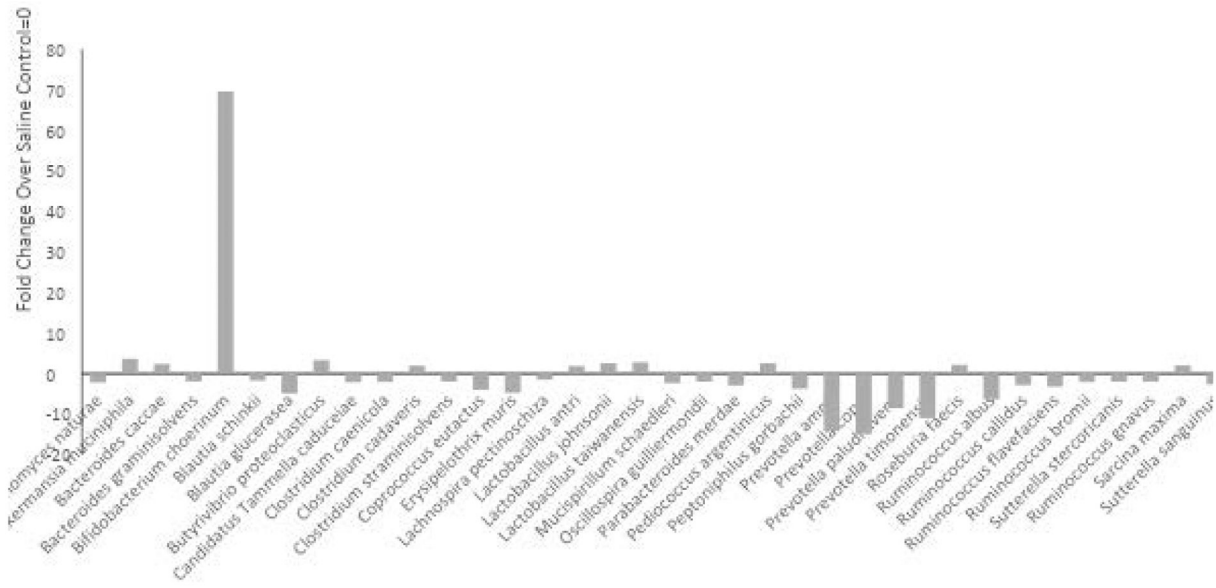

Figure 8:

Effects of Hc-TeTx (60 μg/kg i.m.) on fold change in abundance of gut bacteria species. Values are the fold change over saline control group mean (n=5). Hc-TeTx significantly enriched abundance of 11 species, particularly, Bifidobacterium choerinum, by (a minimum of 69-fold) and 10 others by a minimum of 2-fold. Conversely, Hc-TeTx reduced levels of 24 species by (a minimum of 2-fold) and the levels of Mucispirillum schaedleri by 10-fold. All comparisons are with respect to saline group. There was a total of 611 different species.