The Impact of *Rhodiola Rosea* On Biomarkers of Diabetes, Inflammation, and Gut Microbiota in a Leptin Receptor-Knockout Mouse Model

Mahtab Jafari (✉ mjafari@uci.edu)

University of California, Irvine

Jasmin Grace Juanson Arabit

University of California, Irvine

Robert Courville

University of California, Irvine

Dara Kiani

University of Illinois at Chicago

John M. Chaston

Brigham Young University

Cindy Duy Nguyen

University of California, Irvine

Nilamani Jena

University of California, Irvine

Zhong-Ying Liu

University of California, Irvine

Prasanthi Tata

University of California, Irvine

Richard A. Etten

University of California, Irvine

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Abstract

Type 2 diabetes is the most prevalent endocrine disease in the world, and recently the gut microbiota have become a potential target for its management. Recent studies have illustrated that this disease may predispose individuals to certain microbiome compositions, and treatments like metformin have been shown to change gut microbiota and their associated metabolic pathways. However, given the limitations and side effects associated with pharmaceuticals currently being used for therapy of diabetes, there is a significant need for alternative treatments. In this study, we investigated the effects of a root extract from *Rhodiola rosea* in a Leptin receptor knockout (*db/db*) mouse model of type 2 diabetes. Our previous work showed that *Rhodiola rosea* had anti-inflammatory and gut microbiome-modulating properties, while extending lifespan in several animal models. In this study, treatment with *Rhodiola rosea* improved the insulin response, and significantly decreased serum lipopolysaccharide and C-reactive protein levels. We hypothesize that these changes may in part reflect the modulation of the microbiota, resulting in improved gut barrier integrity and decreasing the translocation of inflammatory biomolecules into the bloodstream. These findings indicate that *Rhodiola rosea* is an attractive candidate for further research in the management of type 2 diabetes.

Introduction

Type 2 diabetes (T2D) is a metabolic disease that currently ranks as one of the largest concerns of global public health, affecting an estimated 476 million people worldwide¹. With both genetic and environmental factors contributing to this complex disease, diabetes is a leading cause of mortality in many countries and globally impacts life expectancy in both developed and developing nations^{1,2}. One of the hallmarks of the disease is hyperglycemia resulting from defects in insulin secretion, insulin action, or both³. Of the three forms of clinical diabetes, the vast majority (about 90%) of patients have T2D, which is characterized by insulin resistance³. Whereas there are many environmental and behavioral factors that modulate genetic susceptibility to this form of diabetes, the prevalence of T2D has risen steadily in recent decades⁴. Due to rising health costs associated with the increasing incidence and prevalence of diabetes worldwide^{5,6}, identifying and evaluating safe and cost-effective therapeutic interventions in the management of T2D is of increasing importance. Current treatment algorithms for T2D include lifestyle changes, as well as oral and parenteral drugs⁷. However, many current treatments have significant limitations or side effects that can impact this large patient population. Although metformin is first-line treatment for T2D, it has side effects including nausea and diarrhea and is contraindicated in patients predisposed to lactic acidosis^{7,8}. Insulin secretagogues such as sulfonylureas and meglitinides tend to lose their efficacy over time due to beta cell failure^{9,10}, while alpha-glucosidase inhibitors, thiazolidinediones, and dipeptidyl peptidase-4 inhibitors have unique mechanisms of action, but adverse effects such as weight gain, heart failure, and gastrointestinal issues have led to poor compliance with therapy^{11–14}. Thus, there is a need for new therapeutic interventions in T2D that are safe and effective.

There is significant evidence that inflammation plays a central role in the pathogenesis of T2D through two distinct pathways¹⁵. Obesity, specifically visceral adiposity, causes systemic inflammation through infiltration of adipose tissue by macrophages^{16,17} and production of pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-6, and CCL2^{18,19}, which act as antagonists of insulin signaling²⁰. Recent studies also suggest that differences in the human gut microbiome between normal and diabetic subjects²¹ are linked to systemic inflammation through altered gut integrity^{22,23}, increased circulating gram-negative bacteria and endotoxin^{22,24}, as well as lipopolysaccharide (LPS)-induced inflammatory cytokine secretion through TLR-4 signaling^{24,25}. Evidence in humans also suggests that certain gut microbiome compositions may predispose individuals to conditions such as obesity and diabetes^{26,27}. As crucial components that moderate host health and physiology, the gut microbiome can contribute to inflammation, alterations of intestinal linings, dyslipidemia, and a wide variety of other changes²³. However, studies also indicate that modulation of the gut microbiome can decrease LPS-induced systemic inflammation in a mouse T2D model, thereby counteracting these changes²⁵. Together, these observations suggest that targeting inflammation through the microbiome could be a novel approach to treating T2D^{17,28}.

Plant-derived therapeutics such as cinnamon and curcumin extracts have been shown to have potential anti-diabetic properties, although their efficacy has not been evaluated in randomized clinical trials^{29,30}. The adaptogenic plant *Rhodiola rosea* is used as a medicinal in traditional medical practices worldwide and has been shown to have anti-inflammatory and gut microbiome-modulating properties^{31–33}. As an adaptogen, *R. rosea* represents an important category of pharmacological substances that are known to aid the body in resisting a wide variety of stressors (i.e. biological, chemical, physical, etc.) to maintain homeostasis and stabilize physiological processes that may be disrupted³². *R. rosea* extract appears to be safe in human studies^{34–36}, which makes it an attractive candidate for the treatment of T2D. *R. rosea* extended lifespan in several animal models including worms, snails, and flies^{37–40}. When tested on a fly model deficient in the insulin receptor substrate *chico*, *R. rosea* still extended lifespan but decreased expression levels of *Drosophila* insulin-like peptide (dILP) 2, 3, and 5 in wild-type flies³⁹, suggesting complex effects on the insulin signaling pathway. However, the effects of this plant extract on an animal model of diabetes have not yet been evaluated.

Here, we investigated the effects of a root extract from *R. rosea* in a Leptin receptor knockout (*db/db*) mouse model of T2D. Leptin is a key adipokine responsible for maintenance of energy homeostasis and body mass, whereas mice lacking the Leptin receptor display hyperphagia and consequently develop obesity, visceral adiposity, hyperglycemia, and hyperinsulinemia^{41–43}. In other mice models, deficiencies in Leptin as well as beta-cell dysfunction have been linked to T2D, suggesting that this adipokine plays a key role in the pathogenesis of diabetes⁴⁴. Like human diabetics, *db/db* mice show augmented expression of the differentiation marker Aldh1a3 and reduced nuclear expression of the transcription factor Nkx6.1, and exhibit hyperglycemia compared to age-matched non *db/db* mouse model⁴⁵. These observations make the *db/db* mouse model suitable for the goals of this study.

Results

Rhodiola rosea improves glucose homeostasis in diabetic db/db mice

To assess the effects of *Rhodiola rosea* on a mouse model of T2D, we treated a cohort of 6 week-old leptin receptor-knockout (*db/db*) mice with an extract of *R. rosea* that was verified for quality based on validated biomarkers (see Methods), administered daily by oral gavage for 4 weeks. Control mice received gavage with water. After 4 weeks, both *R. rosea*-treated and control *db/db* mice developed fasting hyperglycemia, and there were no significant differences between the cohorts in response to an intra-parenteral glucose challenge (glucose tolerance test; Supplementary Fig. 1). Following a week of recovery, we next tested the response of the treatment cohorts to an exogenous insulin challenge (insulin tolerance test) at T5, one week after supplementation had ended. At this time point, the fasting blood glucose levels of the two cohorts were significantly different, with the control gavage group displaying higher levels (Fig. 1). *R. rosea*-treated mice showed a response to insulin challenge, with decreasing blood glucose over a 2 hour period post-injection, while control mice lost their response to insulin after 30 minutes. This suggests that *R. rosea* treatment improves the insulin resistance that is characteristic of *db/db* mice.

Rhodiola rosea does not affect the body mass of db/db mice

We evaluated the body mass of the mice on a weekly basis during the study to assess whether potential changes in blood glucose levels of the *R. rosea* supplemented mice and the controls were due to a decrease in obesity. Although the mean body weight of *R. rosea*-treated *db/db* mice was lower than control water-treated mice in weeks 8 through 14 of the study, these differences were not statistically significant (Fig. 2).

Rhodiola rosea modestly modulates the gut microbiome of db/db mice

To assess the effect of *R. rosea* on the gut microbiome of *db/db* mice, we collected serial stool samples (pretreatment and weeks 1, 2, and 6 on treatment) from females of the two cohorts and analyzed the composition of the microbial community of the gut with 16S rRNA amplicon next-generation sequencing. The microbiome of both cohorts were dominated by *Bacteroidetes* and *Firmicutes*, and most of the reads could be designated as amplicon sequence variants (ASVs) within four major taxonomic assignments: the families *Rikenellaceae* and *S24-7* (*Bacteroidetes*), and the family *Lachnospiraceae* and genus *Lactobacillus* (*Firmicutes*). Of time and *R. rosea* treatment, time accounted for the most variation in the microbiota composition of water and *R. rosea*-treated mice (Fig. 3). When the microbiota composition of the two cohorts was subjected to Bray-Curtis analysis⁴⁶, which does not account for phylogenetic relationships, there was a significant effect of *R. rosea* treatment on the microbiome of *db/db* mice (Fig. 4 and Table 1). The only taxa that varied with treatment or with time and treatment were reads assigned to the *Desulfovibrionales*, clustered at the order level. These were more abundant in *R. rosea*-treated mice than control mice (Fig. 5), including during the time point before *R. rosea* was administered, suggesting this difference may be more strongly attributed to differences in microbiota composition before the experiment began (e.g. early cage effects) than to *R. rosea* treatment. Together, these findings identify

modest but significant variation in the composition of the mouse gut microbiota associated with *R. rosea* treatment.

Table 1
Results of PERMANOVA on Bray-Curtis distances of microbiota data.

	Degrees of freedom	Sum of Squares	Mean Squares	f	R ²	p-value
<i>Treatment</i>	1	0.2	0.2	3.64	0.04	0.02
<i>Time Point</i>	3	2.89	0.96	17.69	0.55	0
<i>Interaction</i>	3	0.35	0.12	2.14	0.07	0.02
<i>Residuals</i>	34	1.85	0.05	NA	0.35	NA
<i>Total</i>	41	5.29	NA	NA	1	NA

Rhodiola rosea **decreases circulating lipopolysaccharide levels and pro-inflammatory cytokines**

To observe whether the changes of the gut microbiota associated with *R. rosea* treatment had any physiological consequences, we determined the LPS content from serum samples from the two cohorts via a limulus amoebocyte lysate (LAL) assay. Treatment with *R. rosea* decreased the lipopolysaccharide (LPS) levels in the serum by almost 50% (Fig. 5). Whereas circulating LPS (derived from gram-negative bacteria) triggers production of inflammatory cytokines by tissue macrophages and other cells, to further observe inflammation levels exhibited by the *db/db* mice, we determined if *R. rosea* treatment was associated with changes in C-reactive protein (CRP), an inflammatory marker exclusively produced by hepatocytes. Similar to LPS, *R. rosea* treatment decreased hepatic CRP transcript expression by about 40% (Fig. 6).

Discussion

The goal of this study was to evaluate the impact of *R. rosea*, a medicinal plant of emerging interest and possible therapeutic value, on the phenotype and the gut microbiota of the *db/db* mouse model of T2D. Although this study shares a common format with a number of published microbiota-disease interaction articles^{47–50}, it is one of the few studies that evaluates the impact of a botanical extract on the gut microbiota throughout the progression of T2D. Although the format of our study was correlative and associative, the results may serve as the basis for future mechanism-based studies.

We elected to use a model with a severe phenotype of diabetes, *db/db* mice, because we wanted to investigate whether *R. rosea* can improve glucose homeostasis in a T2D model that is similar to severe and advanced human T2D in terms of visceral adiposity and insulin resistance. Due to an absence of leptin receptor expression in the brain, the *db/db* mice consume excess calories and develop obesity and insulin resistance, exhibiting hyperglycemia and dyslipidemia⁴³. The use of this severe model may have prevented us from observing beneficial effects of *R. rosea* that might be relevant to less severe

phenotypes of diabetes (i.e., pre-diabetes), but allowed us to study the effects of *R. rosea* in a T2D mouse model without resorting to a high-fat diet or other dietary manipulations.

We observed no significant differences between the two cohorts in fasting blood glucose or in the response to a parenteral glucose challenge (GTT) after 4 weeks of treatment, which is unsurprising given that *db/db* mice have extreme fasting hyperglycemia (> 800 mg/dL) at 10 weeks of age⁴⁵ (Supplementary Fig. 1). However, at T5 (one week after treatment had ended), *R. rosea* significantly lowered the fasting blood glucose of *db/db* mice. This suggests that more prolonged administration of *R. rosea* may modulate the T2D phenotype, either by improving the function of insulin-responsive tissues in *db/db* mice or ameliorating pancreatic beta cell exhaustion that is observed in this model⁴⁵. The *R. rosea*-treated mice also had significantly lower blood glucose levels in response to insulin challenge, suggesting that the extract improves insulin sensitivity in the *db/db* model.

To rule out that the decreases in blood glucose levels were a direct effect of *R. rosea* and not a consequence of decreased food intake and reduction in obesity, we measured the body mass of the *db/db* mice over time. Since there were no significant differences in the body mass, we conclude that *R. rosea* did not affect the hyperphagia associated with the *db/db* phenotype, an observation consistent with other studies demonstrating that *R. rosea* prolongs lifespan through a mechanism distinct from caloric restriction⁴⁷. Therefore, it is plausible that *R. rosea* improves glucose homeostasis in *db/db* mice through other mechanisms.

It has been reported previously that *db/db* mice displays elevated levels of bacteria in the gut from the S24-7 family compared to wild-type populations⁵¹, and that result was observed here, with S24-7 bacteria dominating the composition of the microbiome in both cohorts at all time points. Additionally, the presence of sulfate-reducing bacterial species from the Desulfovibrionales order has been associated with the pathology of T2D²¹. In this study, the only reads that varied across treatments were the reads from the order Desulfovibrionales. However, we were surprised to see higher relative abundance of these bacteria in the *R. rosea* treatment group than in the control group, since it has previously been reported that treatment with salidroside, a glycoside found in *R. rosea*, causes a decrease in Desulfovibrionales levels⁵². Within the group of mice that received *R. rosea* treatments, the abundance of Desulfovibrionales were more abundant than in controls during the first two timepoints, but had comparable abundances between the control and *R. rosea* treatments at timepoints T2 and T6. We cannot explain why the two populations differed at the T0 timepoint, which preceded any treatment, but we observed two possible patterns that are not mutually exclusive. First, the decrease in relative abundance of Desulfovibrionales from T0 to T2 in the *R. rosea* treatment suggests the treatment may affect bacterial abundance. Second, the convergence of Desulfovibrionales abundance in the *R. rosea*-treated and control cohorts at the T2 and T6 time points suggests we may be observing a change in abundance that is determined by shared factors between the groups, such as changes in age.

Multiple studies have demonstrated major changes in the gut microbiota during the development of obesity and T2D in humans^{27,53,54}. When compared to healthy control groups, humans with T2D

exhibited significantly reduced proportions of phylum *Firmicutes* and class *Clostridia*, in addition to compositional changes in the microbiota^{53,54}. These changes may provide a protective advantage to mice that received *R. rosea* as seen by a reduction in downstream inflammatory markers (Fig. 5). It has been reported that the gut integrity depends on the gut microbiota and that the modulation of the gut microbiota may lower intestinal permeability by a mechanism involving glucagon-like peptide-2 (GLP-2)¹⁹. Gut integrity plays an important role in the whole-body homeostasis since its disruption causes metabolic endotoxemia, which is the translocation of LPS from Gram-negative bacteria into the bloodstream from the gut, and has been linked to chronic inflammation¹⁶. Our group has shown that *R. rosea* is not directly influencing the microbial composition through any direct anti-microbial activity³³. In our previous study, the plant extract did not show anti-microbial effects when tested on bacteria isolated from *Drosophila melanogaster* whole-body homogenate³³.

In humans, excess calories have been shown to play a role in the induction of changes to the gut bacterial community⁵⁵. According to a recent report, *db/db* mice display impaired gut barrier integrity compared to wild-type non-diabetic mice⁵⁶. It has also been shown that in mice, high-fat diet-induced microbial dysbiosis led to a decrease in gut integrity and controlled metabolic endotoxemia²⁵. Due to the close relationship between the gut microbiota and gut integrity^{22,25}, we evaluated the LPS levels in the serum of the *db/db* mice. We observed that mice on water gavage had significantly higher levels of LPS in serum compared to the *R. rosea*-treated mice. We postulate that the modulation of the gut microbiota by the plant extract improved gut barrier integrity, thus lowering the rate of translocation of LPS into the bloodstream, which in turn, decreased expression of a hepatic inflammatory marker.

Analysis of the mentioned data led us to postulate that *R. rosea* improved insulin sensitivity of *db/db* mice by modest modulation of the gut microbiota which decreased metabolic endotoxemia and subsequent inflammation. The improvements in fasting blood glucose levels and insulin response may be due to the decreased inflammatory status of the host wherein normal cell processes may have been prioritized instead of stress responses¹⁵.

In conclusion, while previous studies have illustrated that *R. rosea* has anti-inflammatory and gut microbiome-modulating properties which have been reported to extend lifespan in several animal models, the findings of this study present a possible mechanism of action for the plant extract and its effects on a diabetic mouse model. This is important, in part, because the Leptin receptor knockout (*db/db*) mouse model of T2D that was analyzed is based on genetic characteristics—which eliminated the vast array of environmental and behavioral factors that otherwise may have acted as confounding variables. While only a modest modulation of the gut microbiome was indicated in this study, we hypothesize that these changes may have improved the integrity of the gut barrier, thereby accounting for the significant decreases seen in both serum lipopolysaccharide and hepatic C-reactive protein levels. These findings suggest that *R. rosea* is a good candidate for further investigation as a potential treatment for T2D. However, additional research is needed to elucidate if the beneficial effects of *R. rosea* are mediated

through modulation of intestinal epithelial cell integrity, resulting in normal intestinal permeability in the diabetic environment.

Methods

All methods were performed in accordance with applicable guidelines and regulations.

Mice

BKS.Cg-*Dock7^{m +/+}* *Lep^{db}*/J and C57Bl/6 mice were obtained from the Jackson Laboratory (Bar Harbor, ME). *Lep^{db}*/J mice were bred to generate homozygous *Lep^{db}/Lep^{db}* (*db/db*) pups which were used to model T2D. Mice were maintained in 12 hour light/dark and had free access to food and water. Mice were weaned when they were 3 weeks old, and entered into the study at 6 weeks of age.

Study Timeline

The study timeline is summarized in Supplementary Fig. 2. Mice were administered either water (control) or 125 mg/kg *R. rosea* extract (treatment) by oral gavage daily for the 4 weeks of treatment, initiated when the mice were 6 weeks old (T0) and continued until the mice were 10 weeks old (T4). The quality of the *R. rosea* extract was verified by HPLC that showed the extract contained 1.3% salidroside and 3.9% rosavins, consistent with a high-quality extract (Data on File). Fecal samples were collected at 6 weeks of age (T0, prior to treatment), 7 weeks of age (T1), 8 weeks of age (T2) and 12 weeks of age (T6). The Glucose Tolerance Test (GTT) was performed at week 10 and Insulin Tolerance Test (ITT) was performed at week 11. Mice were sacrificed and dissected at Week 14. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of UCI. All experiments were performed in accordance with UCI IACUC guidelines and regulations and with ARRIVE guidelines (<https://arriveguidelines.org>).

Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT)

Given the importance of *in vivo* mouse models for studying the pathogenesis of T2D and various treatment interventions, several methods have been developed to investigate glucose tolerance, as well as the secretion and action of insulin in these models⁵⁷. To ascertain potential differences between treatment and control groups, with regards to glucose homeostasis, both glucose and insulin tolerance tests were performed. Four hours before the assays were performed, the food was removed to induce a state of fasting. Blood samples (~2 µL) were drawn from the mouse tail to record the blood glucose level using an AlphaTrak2 Blood Glucose Monitoring System. The first drop of blood from the mouse tail was wiped away, and the second drop was used to record values. Blood glucose values were recorded 30, 60, and 90 minutes after the injection of either 0.5g/kg interperotinel of 10% glucose (BW x 5 µL) or 0.75 IU/kg ip of 0.25 IU/mL insulin (BW x 3). Data were analyzed using GraphPad Prism 8.

Microbiome analysis

Fecal samples from the mice were collected at 6 weeks of age (T0, prior to treatment), 7 weeks of age (T1), 8 weeks of age (T2) and 12 weeks of age (T6). DNA was extracted by the initiative using the Zymobiomics Mini DNA kit. 16S rRNA amplicon PCR was performed, targeting the V4-V5 region using the EMP primers (515F (barcoded) and 926R)⁵⁸. The samples were prepared into a library that was sequenced at the UC Irvine Genomics High Throughput Facility on an Illumina MiSeq, using paired-end 300 bp v3 sequencing chemistry. The raw sequence data were imported into QIIME2 (qiime2.org)^{59,60} and demultiplexed⁵⁸. This bioinformatics platform has recently been rewritten and reengineered for the next generation of microbiome sequencing, facilitating taxonomic and phylogenetic analyses. While dozens of software packages written in various programming languages are often needed for comprehensive analyses of this type of sequencing data, QIIME2 allows for “sequence quality checking, denoising, taxonomic classification, alignment, and phylogenetic tree building”—allowing for seamless analysis, description, and quantification of microbial communities⁵⁸.

The sequences were assigned a taxonomic classification using the q2-feature-classifier⁶¹ on a GreenGenes database downloaded in July 2020 from the QIIME2 website (qiime2.org)⁶². Because the quality scores of the data were low, no read pairs passed DADA2 quality filter steps⁶³. Therefore, we analyzed the data only using the forward reads, which had higher average quality scores than the reverse reads. Forward reads that passed quality filtering and denoising by default DADA2 parameters in QIIME2 were rarefied to 11,390 reads per sample and beta-diversity distance metrics were calculated using QIIME2^{64,65,46}. As part of calculating the Unifrac beta-diversity metrics we constructed a phylogenetic tree with fasttree2⁶⁶ based on mafft alignment⁶⁷. PERMANOVA⁶⁸ and ANCOM⁶⁹ analyses were performed in R.

LAL assay of blood serum

Serum samples were diluted 40-fold and were evaluated using the PierceTM Chromogenic Endotoxin Quant Kit according to the manufacturer’s recommended protocol. Data were analyzed using GraphPad Prism 8.

Body Mass Assay

The body mass of mice from both treatments was assayed from weeks 8-14 to test for obesity related differences. Data from male and female subjects were pooled, and Mann-Whitney tests at each timepoint indicated there were no significant differences in body weights. Data were analyzed using GraphPad Prism 8.

Gene Expression Assay

RNA was extracted from the liver tissue using Trizol. The RNA extract was treated with DNA-freeTM DNA Removal Kit from Invitrogen to remove any contaminating DNA. A cDNA library was generated with the iScriptTM cDNA Synthesis Kit from Bio-Rad. qPCR was performed on a BioRadMJ Mini Personal Thermal Cycler with iQ SYBR Green Supermix. The PCR amplification program consisted of an initial denaturation

set at 94°C for 3 minutes, followed by 40 three-step cycles at 94°C for 10 seconds, 60°C at 30 seconds and at 72°C for 45 seconds. The $\Delta\Delta C_t$ Method was used to quantify the relative expression of genes of interest⁴⁶. The CRP (gene of interest) and GAPDH (reference gene) primers were derived from PrimerBank (Supplementary Table 1). Data were analyzed using GraphPad Prism 8.

Declarations

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AUTHOR CONTRIBUTIONS

Mahtab Jafari

As the primary investigator, Dr. Jafari designed and performed experiments and oversaw the data analysis (except for the microbiome data analysis). She also wrote the manuscript.

Jasmin Grace Juanson Arabit

As an undergraduate student in Jafari Lab, Ms. Arabit participated in the experiments and coordinated the work.

Robert Courville

As a graduate student in Jafari Lab, Mr. Courville was involved in the microbiome data analysis, revising sections of the manuscript, and reference management.

Dara Kiani

As an undergraduate student in Jafari Lab, Mr. Kiani was involved in collecting and handling the microbiome samples and make recommendations on the microbiome samples data analysis.

John M. Chaston

As a research collaborator, Dr. Chaston analyzed the microbiome data, developed the graphs and participated extensively in writing the results and discussion on microbiome data.

Cindy Duy Nguyen

As an undergraduate student in Jafari Lab, Ms. Nguen participated in two of the mouse experiments.

Nilamani Jena

As a project scientist in Dr. Van Etten lab, Dr. Jena, participated in maintaining the mouse colonies and in mouse experiments (injections, blood draws, etc.)

Zhong-Ying Liu

As the lab manager in Dr. Van Etten lab, Dr. Liu, participated in maintaining the mouse colonies and in mouse experiments (injections, blood draws, etc.)

Prasanthi Tata

As a research assistant in Dr. Van Etten lab, Ms. Tata, participated in mouse experiments (injections, blood draws, etc.) and provided feedback.

Richard A. Van Etten⁴

As a research collaborator, Dr. Van Etten helped designed the mouse experiments and provided scientific advice on all the experiments. He also participated in revising and finalizing the manuscript.

COMPETING INTERESTS

The author(s) declare no competing interests.

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Figures

Figure 1

Improved insulin tolerance in *R. rosea*-treated *db/db* mice. Insulin tolerance test of *db/db* mice. * $P < 0.05$, ** $P < 0.01$, multiple t-tests; $n = 7$ for water and $n = 11$ for *R. rosea*.

Figure 2

No effect of *R. rosea* on body weight of *db/db* mice. Body mass measurements of *db/db* mice. No significant differences were found (Mann-Whitney tests at each time point, n=7 for water and n=11 for *R. rosea* treatment).

Figure 3

Effects of *R. rosea* on the gut microbiome of *db/db* mice. A) Analysis of abundance shows composition is dominated by bacteria from the *Bacteroidetes* phylum, especially from the *S24-7* family. B) Bray-Curtis principal coordinate ordination. The corresponding PERMANOVA (Table 1) identifies significant differences in microbiota composition with treatment and time.

Figure 4

Treatment differences in fractional abundance of bacteria from the *Desulfovibrionales* order at each timepoint sampled. Higher abundances were observed in mice treated with *R. rosea* than the control at pretreatment and one week following treatment. No statistically significant differences were observed at the T2 and T6 timepoints.

Figure 5

R. rosea treatment reduces circulating LPS. LAL assay of mouse serum. $P < 0.01$, Mann-Whitney test; $n = 8$ for water and $n = 6$ for *R. rosea*.

Liver CRP Gene Expression

Figure 6

R. rosea decreases hepatocyte CRP expression. Relative expression of CRP transcripts in mouse liver calculated using the DDCT method. $n=8$ for water and $n=6$ for *R. rosea*, $*P < 0.05$.

Supplementary Files

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