Feiolix: Relevant Studies for Inflammation Weight Management & Metabolic Syndrome

INTRODUCTION

Obesity, metabolic syndrome, and diabetes have become major global health concerns. Type-2 diabetes is the most common form of diabetes and accounts for 90% of all cases. Studies have demonstrated that Type-2 diabetes increases the risk of cardiovascular morbidity and mortality.

Maintaining blood sugar levels is important for the general population. Elevated blood glucose causes elevated triglycerides, this can lead to an increased risk of heart disease, storing of unwanted fat, and increases the rate of aging. Weight loss also becomes easier when blood glucose levels are controlled.

Feiolix extract is a high quality, polyphenolic-rich feijoa fruit extract made entirely from New Zealand feijoa. In clinical and pre-clinical studies, Feiolix has been shown to:

- Significantly decrease body weight gain, epididymal adipose tissue weight and non-esterified fatty acids (free fatty acids) *in vivo*;
- Significantly decrease the production of inflammatory cytokines IL-4, TNF-α, and TNF-β *in vivo* (Section 1.3);
- Significantly inhibit the secretion of bacterial lipopolysaccharide induced inflammatory cytokines TNF-α and IL-1β *in vitro* (Section 2.1);
- Significantly inhibit superoxide generation in PMA activated neutrophils *in vitro* (Section 2.1).

This document summarises the following relevant studies on the Feiolix extract control of blood glucose, weight management, immune function and metabolic syndrome.

It is expected that the Feiolix whole fruit powder containing equivalent levels of bioactives plus the cell wall xyloglucans which increase *Bacteroides* levels and activity, producers of the postbiotic propionate which reduces liver fatty acid synthesis gene expression, will maintain or exceed the activities reported here. Unpublished preclinical data using STZ diabetic mice support this hypothesis.

1. In vivo studies

1.1. The effect of feijoa extract on obesity in leptin-deficient obese animal model.

[Published in PCT patent specification: International application # PCT/IB2013/054727]

1.2. The effect of feijoa extract on immune function in aged mice model.

[Published in PCT patent specification: International application # PCT/IB2013/054727]

1.3. Determination of the effect of feijoa extract in high fat diet (HFD) induced metabolic syndrome in C57BL/6J mice.

[Unpublished, study completed May 2018]

2. In vitro

2.1. Evaluation of the anti-inflammatory properties of feijoa extracts.

[Unpublished, study completed August 2015]

1. IN VIVO STUDIES

1.1. THE EFFECT OF FEIJOA EXTRACT ON OBESITY IN LEPTIN-DEFICIENT OBESE ANIMAL MODEL [PUBLISHED IN PATENT SPECIFICATION]

1.1.1. Summary Notes

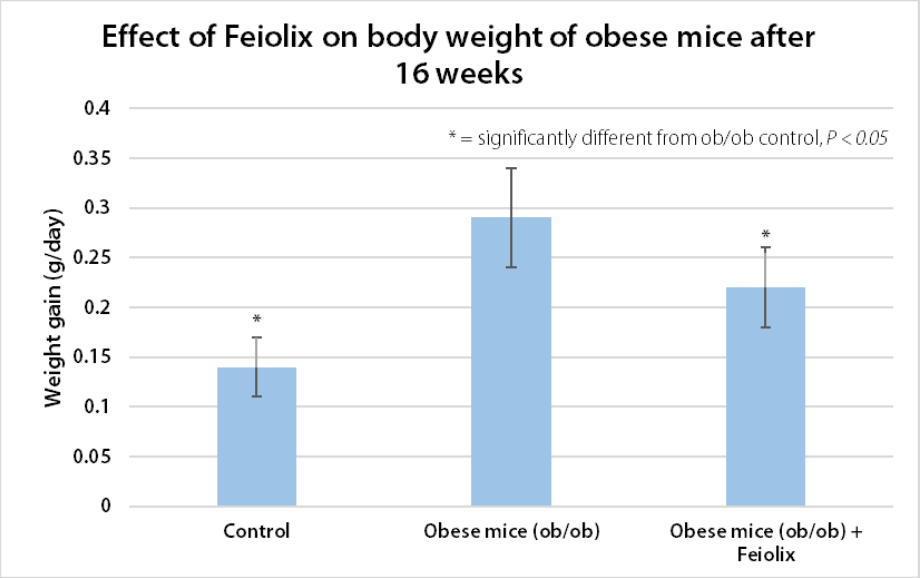
- Leptin-deficient obese (ob/ob) mice were fed a diet supplemented with feijoa fruit extract at a rate of approximately 1.5 mg/mouse/day for 16 weeks (equiv. to 280 mg human dose).
- Food consumption was measured daily, and body weights were measured weekly.
- The lipid profiles in the serum and liver and the weight of organs and adipose tissue were determined after 16 weeks.
- Feijoa fruit extract supplementation significantly decreased the amount of weight gained by 24% when compared to the control in ob/ob mice.
- The obese mice in both the ob/ob control group and feijoa extract supplemented group consumed similar volumes of food.
- The food efficiency ratio significantly decreased in feijoa supplementation group compared to obese control group.
- In mice supplemented with feijoa fruit extract, the weight of the liver was 10.6 % less than that of the control group.
- In order to examine the effect of feijoa supplementation on body fat accumulation, the weight of adipose tissue in ob/ob mice was measured. The weight of epididymal adipose tissue in the feijoa extract group was significantly lower than the ob/ob control group.
- Supplementation in the diet of obese mice with the feijoa extract was found to reduce the hepatic total cholesterol level compared to the ob/ob control mice.
- Obese mice fed a diet supplemented with feijoa extract did not gain as much body weight, show a lower food efficiency ratio, and exhibit significant beneficial changes in hepatic total cholesterol levels.
- The study showed that feijoa extract may physically affect body weight gain and reduce fat tissue accumulation.

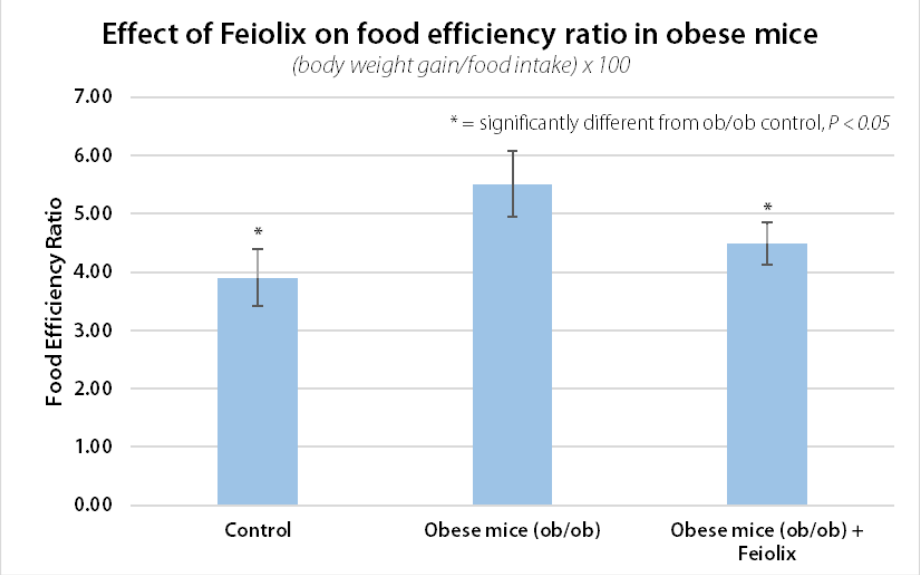
1.1.2. Results

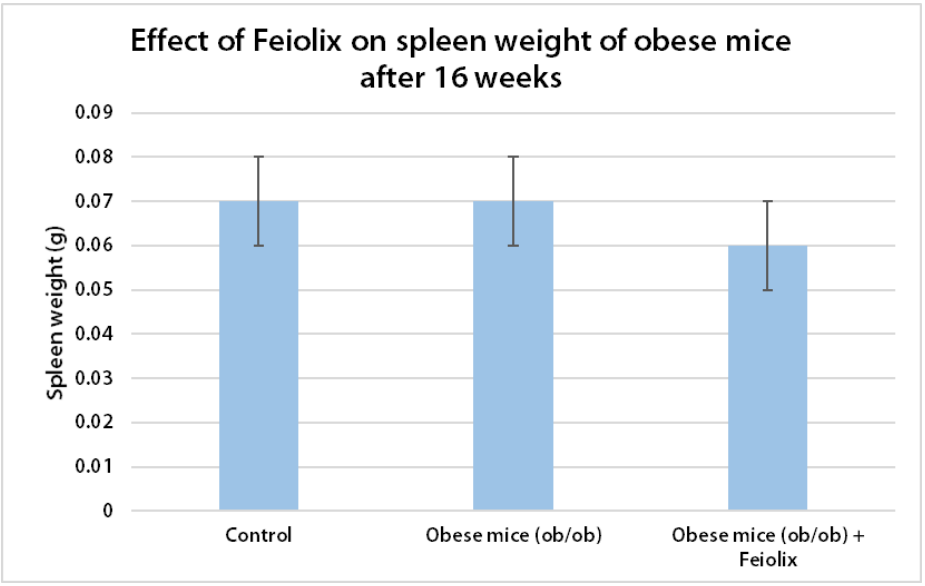
	Normal mice control	Ob/ob control (obese)	Ob/ob mice + Feijoa extract
Change in body weight and food consumption			
Initial body weight (g)	21.79 ± 1.09*	32.45 ± 2.15	34.40 ± 1.96
Final body weight (g)	37.29 ± 3.11*	65.23 ± 3.97	59.05 ± 2.72*
Weight gain (g/day)	0.14 ± 0.03*	0.29 ± 0.05	0.22 ± 0.04*
Food efficiency ratio (body weight gain/food intake) x100	3.90 ± 0.49*	5.51 ± 0.57	4.49 ± 0.37*
Weight of organs and adipose tissues			
Kidney weight (g)	0.22 ± 0.02*	0.27 ± 0.02	0.25 ± 0.02
Liver weight (g)	1.89 ± 0.17*	4.44 ± 0.39	3.97 ± 0.31*
Spleen weight (g)	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01

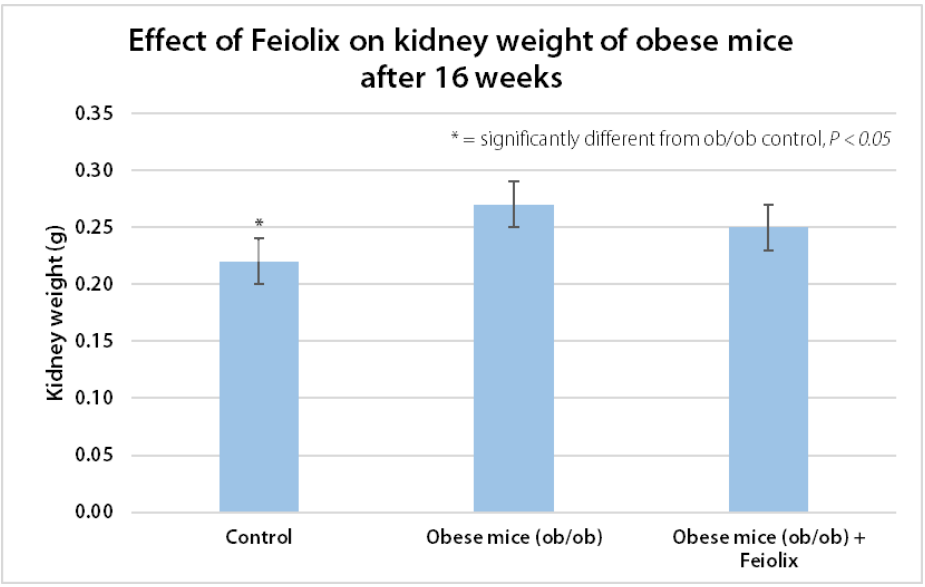
Retroperitoneal adipose tissue weight (g)	0.53 ± 0.19*	3.15 ± 0.46	2.63 ± 0.36
Epididymal adipose tissue weight (g)	1.58 ± 0.26*	4.23 ± 0.36	3.63 ± 0.43*
Serum lipid profile			
Total Cholesterol (mg/dl)	154.67 ± 37.39*	415.36 ± 29.79	386.04 ± 41.42
Triglyceride (mg/dl)	77.57 ± 15.07*	142.53 ± 17.16	121.28 ± 22.17
Hepatic lipid profile			
Total Cholesterol (mg/dl)	179.15 ± 9.85*	453.64 ± 26.44	413.59 ± 18.76*
Triglyceride (mg/dl)	116.03 ± 11.87*	266.73 ± 31.91	229.76 ± 32.02

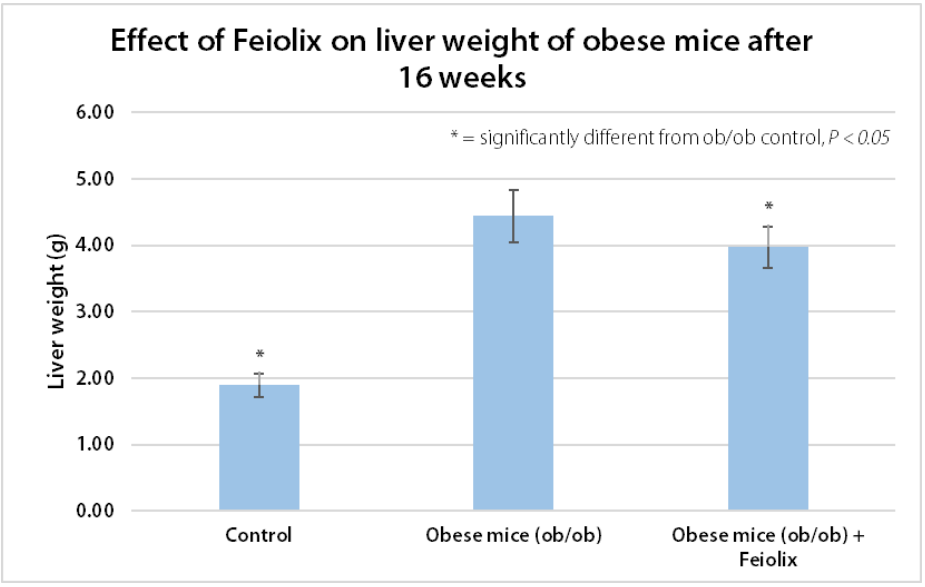
Values are means ± SD from 6 mice/ group. Mean with * indicates a significant difference at $p < 0.05$, compared to negative control.

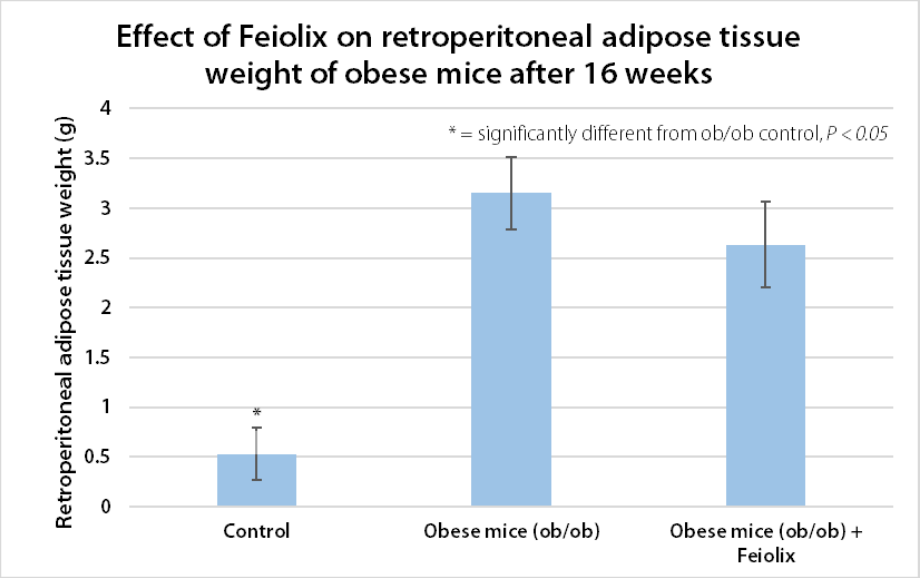


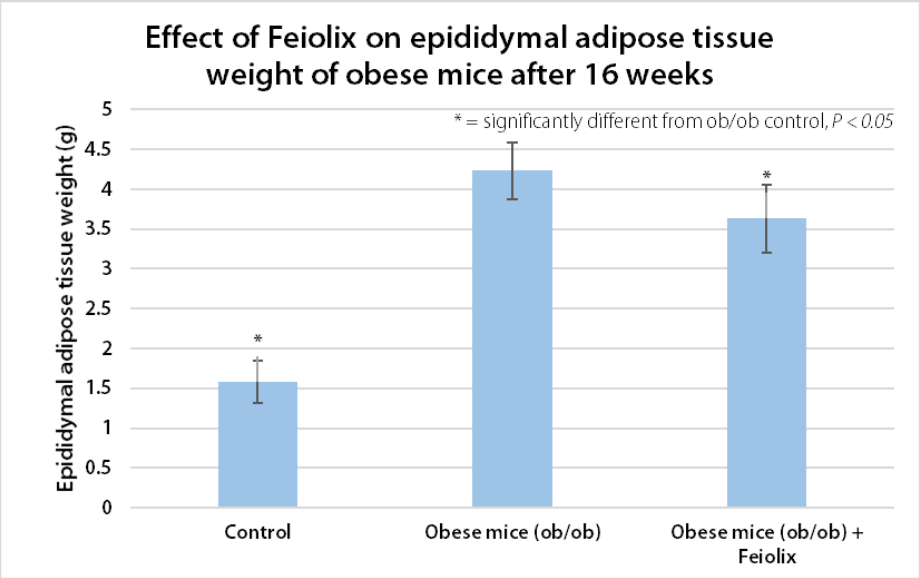


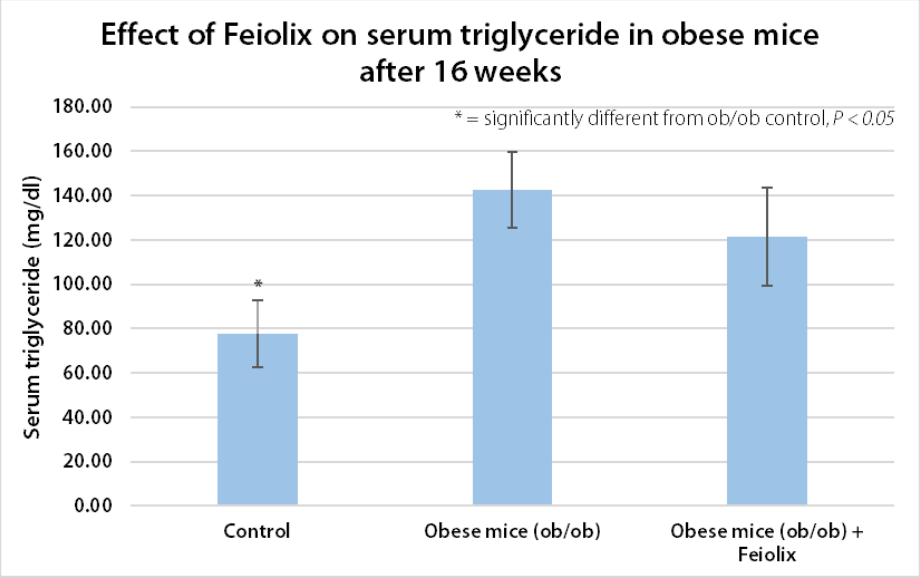

Effect of Feiolix on spleen weight of obese mice after 16 weeks
Spleen weight (g)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Control
Obese mice (ob/ob)
Obese mice (ob/ob) + Feiolix

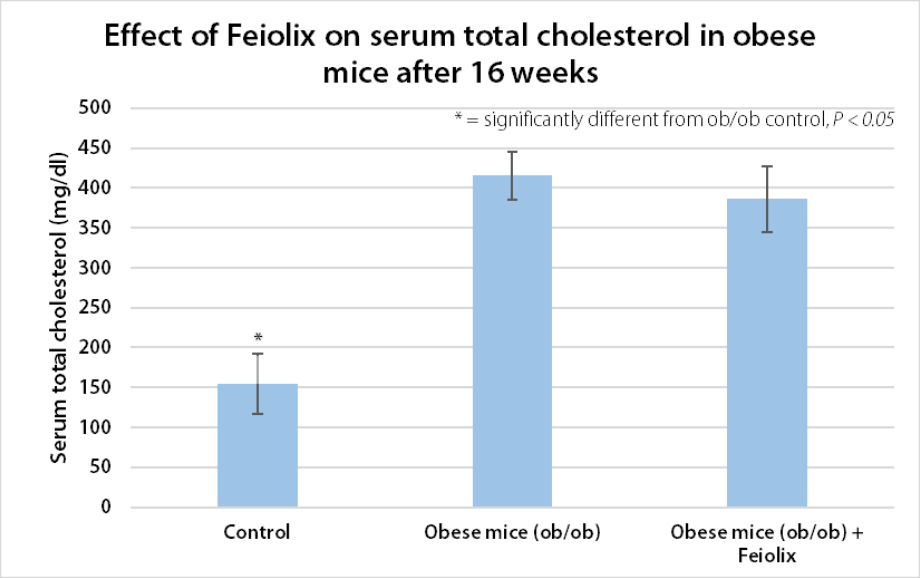

Effect of Feiolix on kidney weight of obese mice after 16 weeks
* = significantly different from ob/ob control, P < 0.05
Kidney weight (g)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
*
Control
Obese mice (ob/ob)
Obese mice (ob/ob) + Feiolix

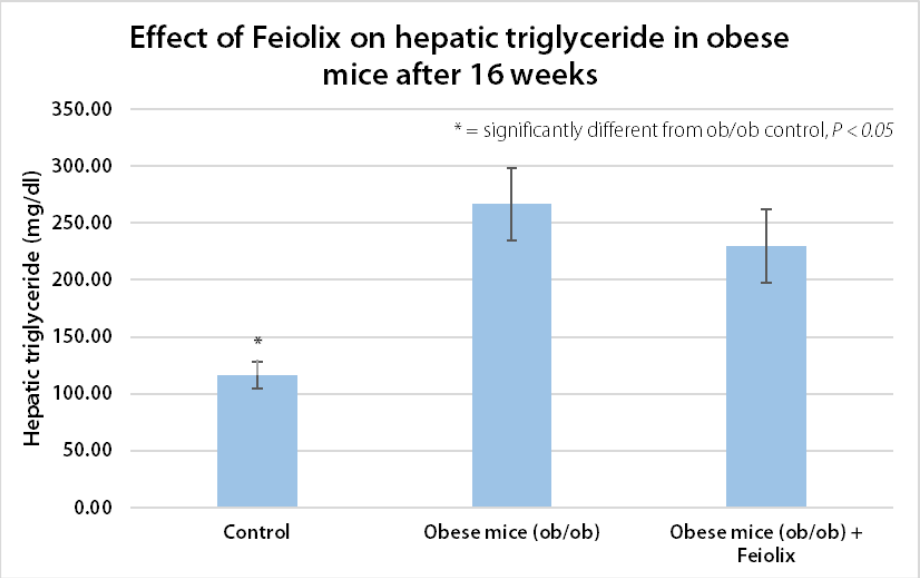

Effect of Feiolix on liver weight of obese mice after 16 weeks
* = significantly different from ob/ob control, P < 0.05
Liver weight (g)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
*
*
Control
Obese mice (ob/ob)
Obese mice (ob/ob) + Feiolix

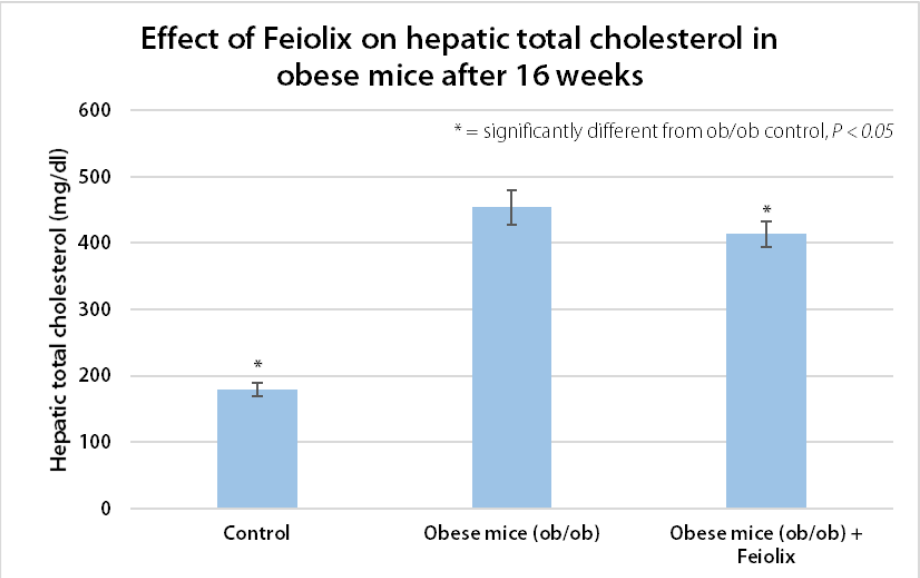

Effect of Feiolix on retroperitoneal adipose tissue weight of obese mice after 16 weeks
* = significantly different from ob/ob control, P < 0.05
Retroperitoneal adipose tissue weight (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
*
Control
Obese mice (ob/ob)
Obese mice (ob/ob) + Feiolix


Effect of Feiolix on epididymal adipose tissue weight of obese mice after 16 weeks
* = significantly different from ob/ob control, P < 0.05
Epididymal adipose tissue weight (g)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
Control
Obese mice (ob/ob)
Obese mice (ob/ob) + Feiolix


Effect of Feiolix on serum triglyceride in obese mice after 16 weeks
* = significantly different from ob/ob control, P < 0.05
Serum triglyceride (mg/dl)
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
*
Control
Obese mice (ob/ob)
Obese mice (ob/ob) + Feiolix


Effect of Feiolix on serum total cholesterol in obese mice after 16 weeks
* = significantly different from ob/ob control, P < 0.05
Serum total cholesterol (mg/dl)
500
450
400
350
300
250
200
150
100
50
0
*
Control
Obese mice (ob/ob)
Obese mice (ob/ob) + Feiolix


Effect of Feiolix on hepatic triglyceride in obese mice after 16 weeks
* = significantly different from ob/ob control, P < 0.05
Hepatic triglyceride (mg/dl)
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
*
Control
Obese mice (ob/ob)
Obese mice (ob/ob) + Feiolix


Effect of Feiolix on hepatic total cholesterol in obese mice after 16 weeks
* = significantly different from ob/ob control, P < 0.05
Hepatic total cholesterol (mg/dl)
600
500
400
300
200
100
0
*
*
Control
Obese mice (ob/ob)
Obese mice (ob/ob) + Feiolix

1.2. DETERMINATION OF THE EFFECT OF FEIJOA EXTRACT IN HIGH FAT DIET (HFD) INDUCED METABOLIC SYNDROME IN C57BL/6J MICE [UNPUBLISHED]

1.2.1. Summary Notes

- Elevated fasting glucose and triglycerides are the key factors affected in diabetes mellitus apart from many other changes affecting many tissues of the body.
- Male mice (C57BL/6J) mice were with standard diet or high fat diet (HFD) supplemented with Feiolix extract for 20 weeks (8 weeks for the induction of metabolic syndrome using HFD, followed by 12 weeks supplementation with Feiolix extract).
- It is widely used and acceptable model because disease pathology is similar to what is observed in humans.
- Experimental groups:

Group	Treatment Details	Diet & Dose (mg/kg body weight per day)	Mice per group
1	Control	Standard diet, 10 Kcal 4% fat	10
2	Disease control	High fat diet, 60 Kcal, 35% fat	15
4	HFD + Feiolix mid dose	High fat diet, 60 Kcal, 35% fat + Feiolix 0.3 mg/20 g mouse (equiv. to 75 mg human dose of the extract)	15
5	HFD + Feiolix high dose	High fat diet, 60 Kcal, 35% fat + Feiolix 0.7 mg/20 g mouse (equiv. to 150 mg human dose of the extract)	15

- When the mice became over weight (~8 weeks), fasting blood glucose levels were measured to confirm hyperglycaemia.
- Mice with high blood sugar (≥130 – 180 mg/dl) levels were chosen for the Feiolix intervention experiments.
- Key markers of metabolic syndrome such as fasting blood glucose, oral glucose tolerance test (OGTT), insulin tolerance test (ITT), insulin resistance (HOMA-IR), free fatty acids, triglycerides and cholesterol, were determined after 12 weeks intervention. Body weight and liver index were also recorded.

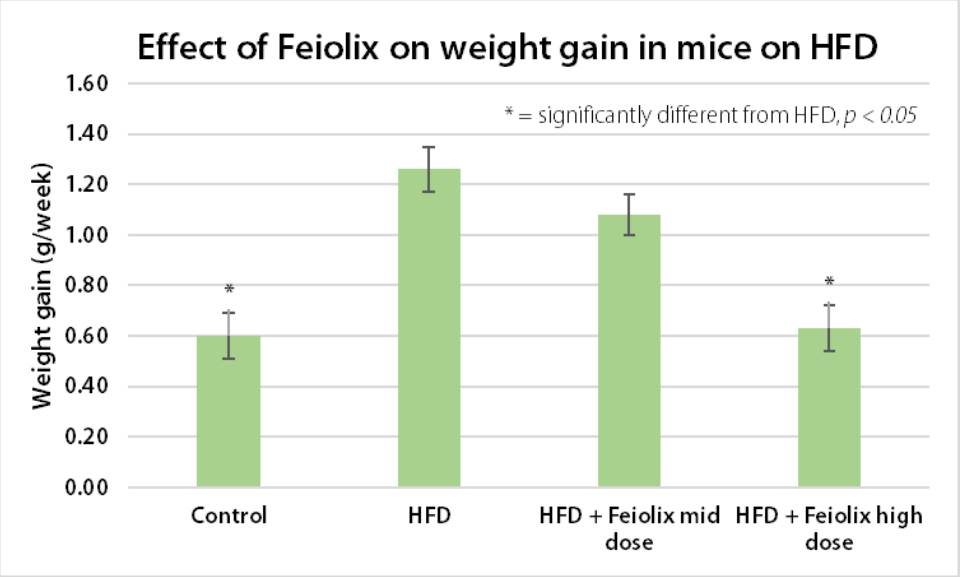
Measurement	Effect of Feiolix
Body weight	– Mice fed with HFD had significant weight gain compared to control. – Feiolix treatment at the mid and high dose showed significant decrease compared to HFD group by the end of the experiment at Week 20 (after 12 weeks treatment).
Liver Index	– Liver index (liver weight/body weight x 100) was high in mice fed with HFD. – Feiolix treatment at the high dose showed significant decrease compared to HFD group.
Non-esterified Fatty Acids (free fatty acid)	– Mice fed with HFD showed significantly elevated levels of free fatty acids. – Feiolix treatment at the mid and high dose showed significant decrease compared to HFD group.
Total cholesterol	– Mice fed with HFD showed significantly elevated levels of cholesterol. – Feiolix treatment at the high dose showed significant decrease compared to HFD group.
Tri-glyceride	– Mice fed with HFD showed significantly elevated levels of triglycerides.

	– Feiolix treatment at the mid and high dose showed significant decrease compared to HFD group.
Fasting glucose	– Mice fed with HFD had significantly higher fasting glucose levels than the control group. – Feiolix treatment at the high dose significantly reduced fasting glucose.
Insulin resistance: HOMA-IR	– Insulin resistance was increased in mice fed with HFD. – Feiolix treatment at the mid and high dose showed significantly decrease in HOMA-IR.
Oral Glucose Tolerance Test (OGTT)	– Glucose administration to the HFD fed animals resulted in increased blood glucose levels compared to control. – Feiolix treatment at the high dose significantly reduced blood glucose level after glucose challenge compared to mice fed with HFD only.
Insulin Tolerance Test (ITT)	– The Insulin Tolerance Test was designed to determine the sensitivity of insulin receptors in tissue by measuring blood glucose levels before and after insulin administration through the intra-peritoneal route. – Insulin administration resulted in higher blood glucose levels in HFD mice than control. – Feiolix treatment at the high dose significantly decreased blood glucose levels, this effect was seen at Week 16 and Week 20 (8th week and 12th weeks after Feiolix treatment respectively).

1.2.2. Results

Body Weight

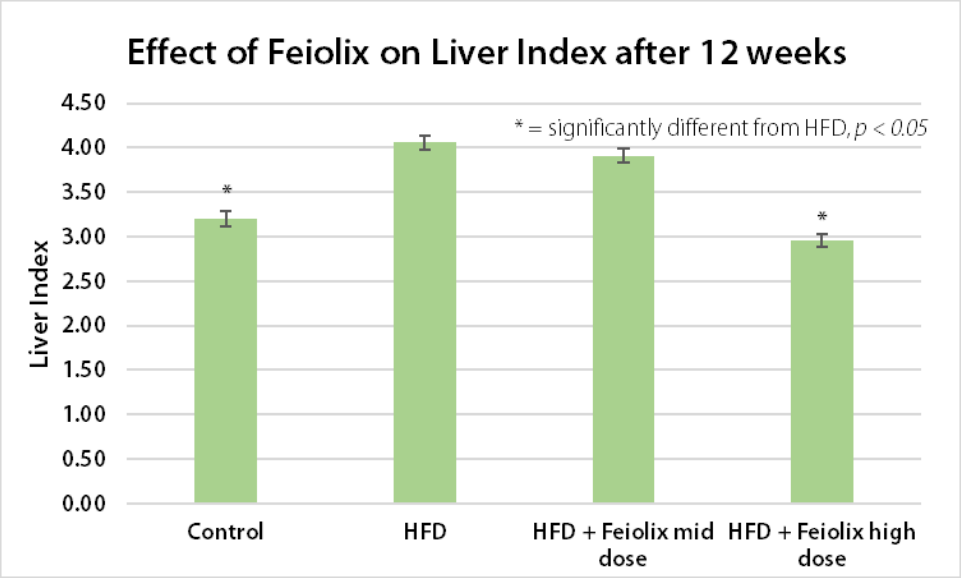
Treatment Group	Bodyweight (g) at Week 20		Change in body weight of individuals (gain as slope in g per week from W 8 to W 20)	
	Mean	SEM	Mean	SEM
Control	37.60^{c}	0.50	0.60^{g}	0.090
HFD	51.84^{d}	0.45	1.26^{h}	0.090
HFD + Feiolix mid dose	50.18^{e}	0.43	1.08^{h}	0.080
HFD + Feiolix high dose	44.61^{f}	0.41	0.63^{g}	0.090



Liver Index

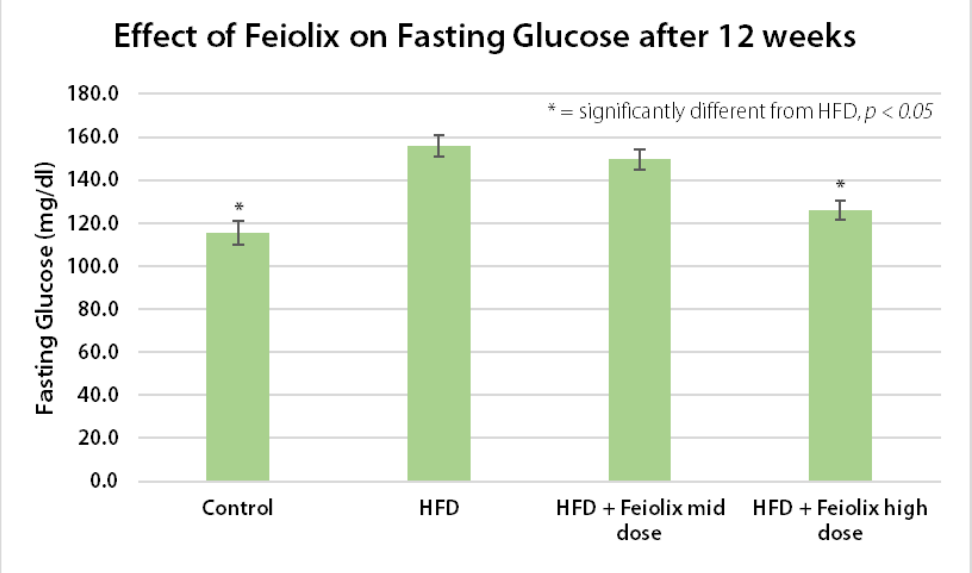
Liver tissues were collected at the end of the experiment and fixed in 10% neutral buffered formalin solution, processed and embedded in paraffin wax. The Liver Index is expressed as = 100(Liver weight/Body weight).

Treatment Group	Mean	SEM
Control	3.20[a]	0.091
HFD	4.06[b]	0.082
HFD + Feiolix mid dose	3.91[bc]	0.078
HFD + Feiolix high dose	2.96[d]	0.075



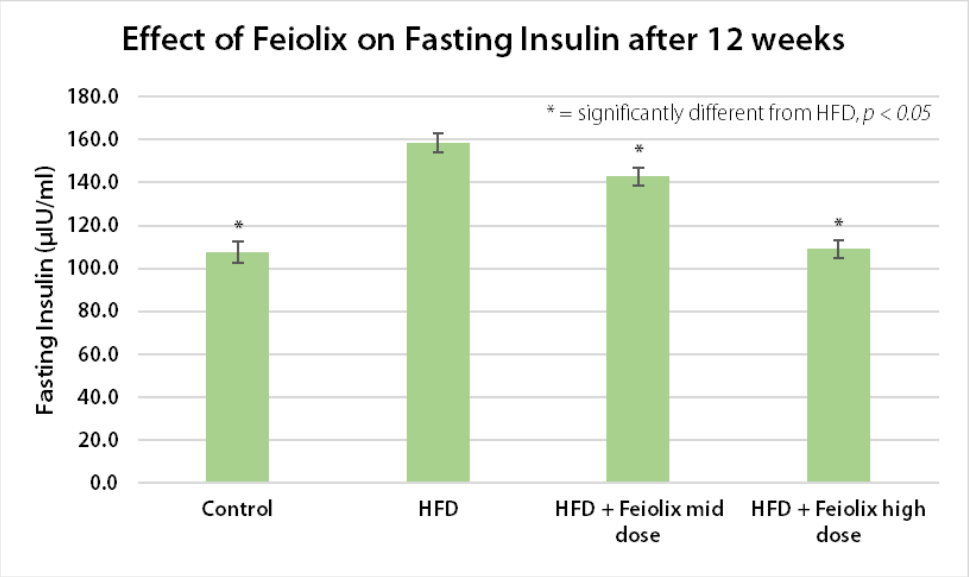
Fasting Glucose

Treatment Group	Fasting glucose at Week 20 (mg/dl)	
	Mean	SEM
Control	115.3[c]	5.46
HFD	156.0[d]	4.88
HFD + Feiolix mid dose	149.7[d]	4.66
HFD + Feiolix high dose	125.8[c]	4.46



Fasting Insulin

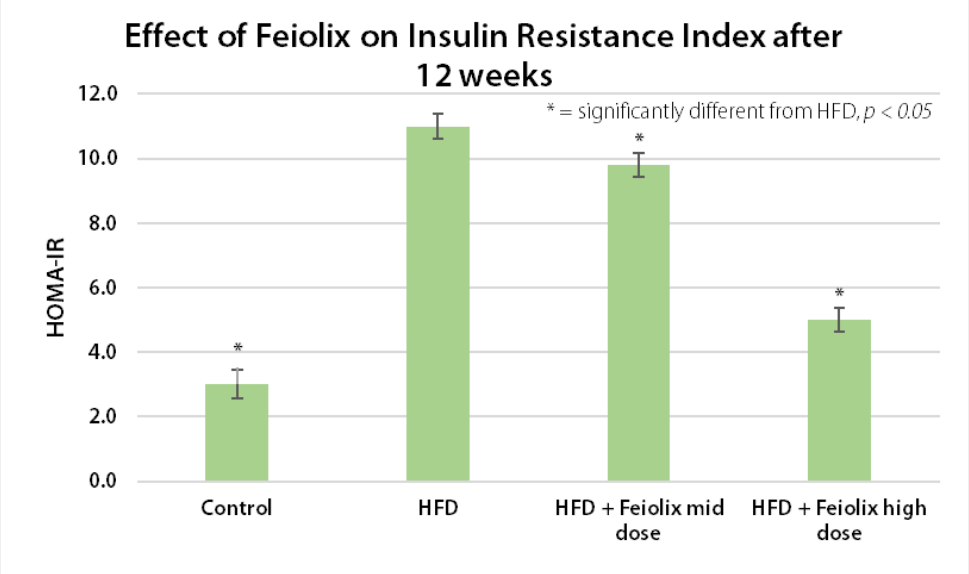
Treatment Group	Fasting glucose at Week 20 (mg/dl)	
	Mean	SEM
Control	107.5[g]	4.96
HFD	158.7[h]	4.43
HFD + Feiolix mid dose	142.9[k]	4.23
HFD + Feiolix high dose	109.0[g]	4.05



Insulin Resistance Index (HOMA-IR) at Week 20

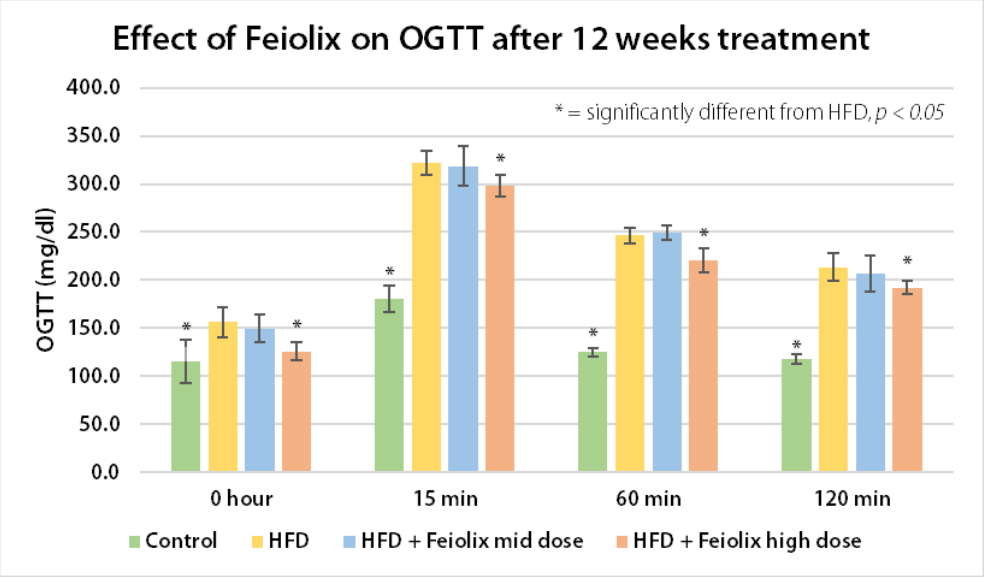
Insulin Resistance Index: The homeostatic model assessment (HOMA) is a measure of insulin resistance observed in metabolic syndrome. It was calculated by using the following formula: fasting insulin µM/L x fasting glucose mmol/L/22.5.

Treatment Group	Insulin (µIU/ml)		Insulin Resistance Index (HOMA-IR)	
	Mean	SEM	Mean	SEM
Control	11.0[a]	1.08	3.0[e]	0.45
HFD	29.4[bc]	0.96	11.0[f]	0.40
HFD + Feiolix mid dose	27.1[c]	0.92	9.8[g]	0.38
HFD + Feiolix high dose	19.5[d]	0.88	5.0[h]	0.37



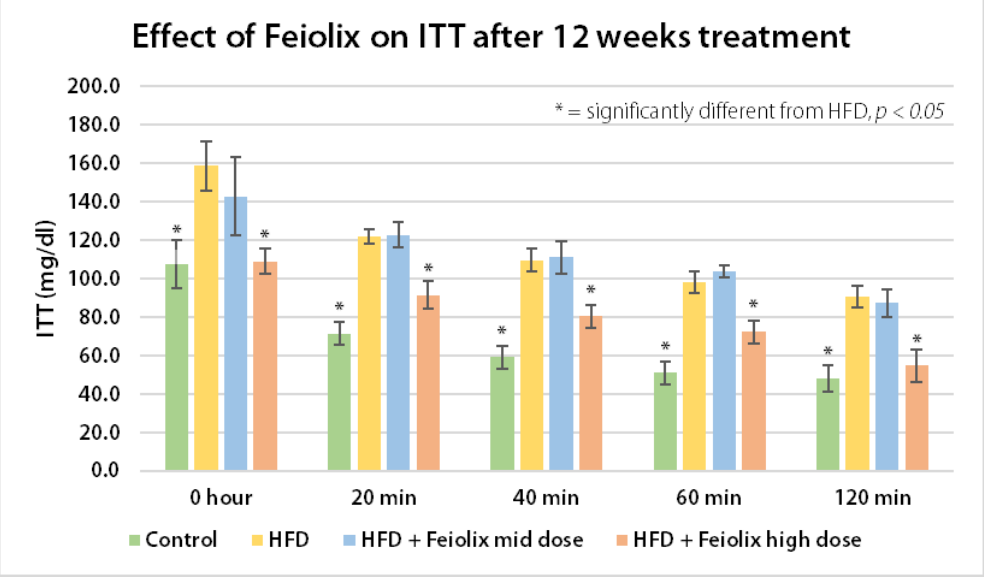
Oral glucose tolerance test (OGTT) (at Week 20)

Treatment Group	0 hour		15 min		60 min		120 min	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	115.2[d]	22.1	180.7[d]	13.6	124.8[i]	4.2	117.6[l]	5.2
HFD	156.0[b]	15.2	322.3[e]	12.6	246.5[j]	8.2	213.5[m]	14.2
HFD + Feiolix mid dose	149.7[b]	14.3	318.7[e]	21.1	249.4[j]	7.2	206.8[m]	18.5
HFD + Feiolix high dose	125.8[c]	9.2	297.9[f]	11.1	220.6[k]	12.2	192.3[n]	7.4



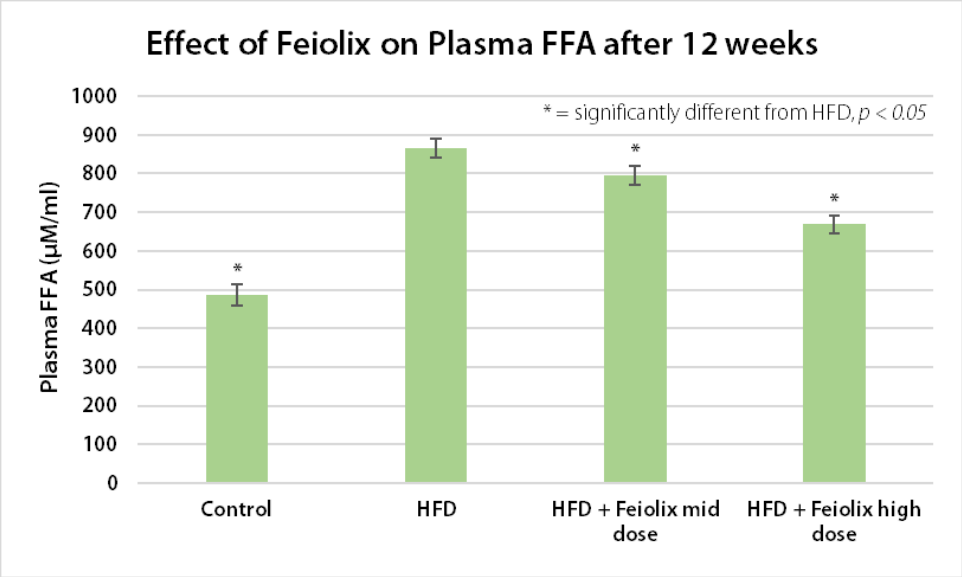
Insulin tolerance test (ITT) (at Week 20)

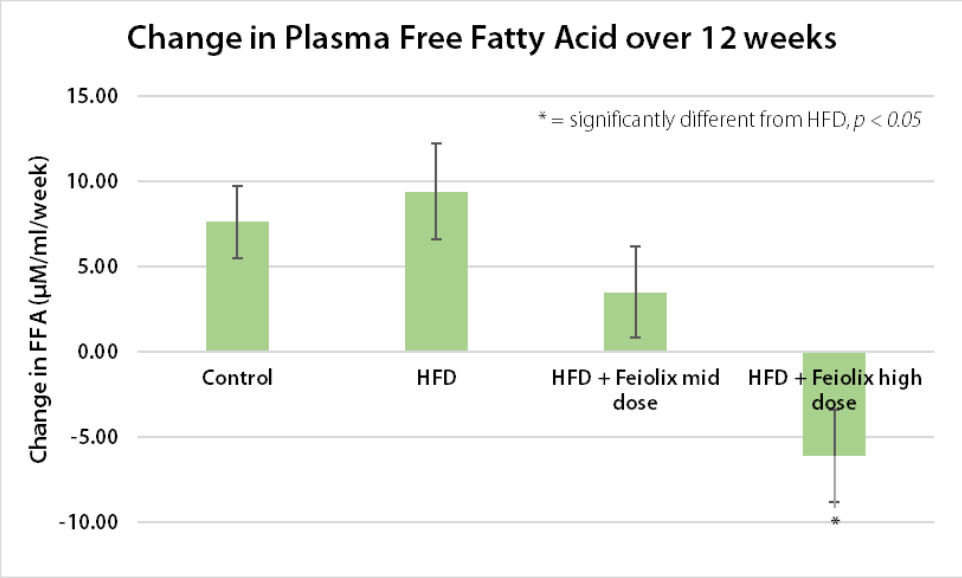
Treatment Group	0 hour		15 min		60 min		120 min	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	115.2[d]	22.1	180.7[d]	13.6	124.8[i]	4.2	117.6[l]	5.2
HFD	156.0[b]	15.2	322.3[e]	12.6	246.5[j]	8.2	213.5[m]	14.2
HFD + Feiolix mid dose	149.7[b]	14.3	318.7[e]	21.1	249.4[j]	7.2	206.8[m]	18.5
HFD + Feiolix high dose	125.8[c]	9.2	297.9[f]	11.1	220.6[k]	12.2	192.3[n]	7.4



Non-esterified Free Fatty Acid

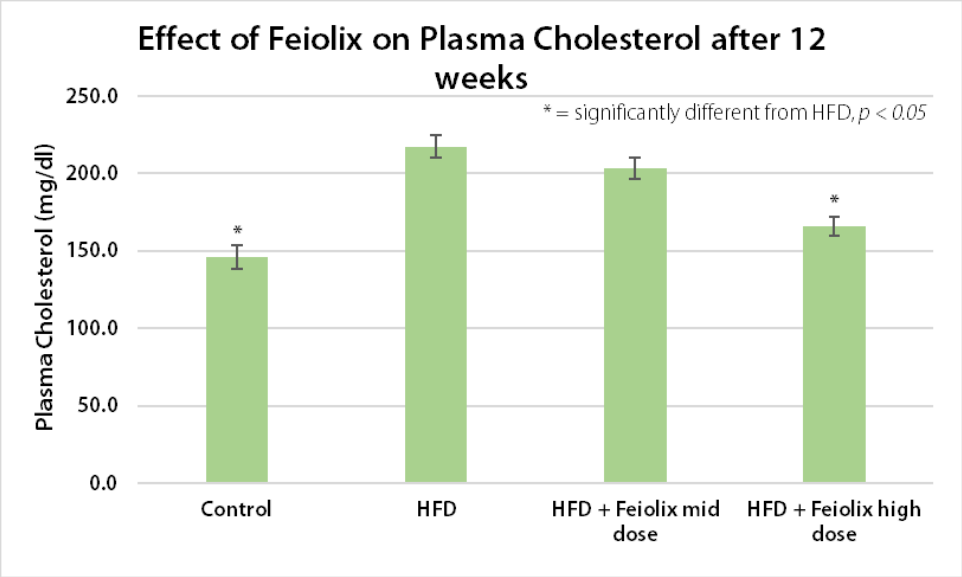
Treatment Group	Baseline NEFA at Week 20		Change in baseline NEFA of individuals (change as slope per week (µM/ml) from W 8 to W 20)	
	Mean	SEM	Slope (µM/ml/week)	SE of the slope
Control	487[a]	28.0	7.6[a]	2.1
HFD	866[b]	25.1	9.4[a]	2.8
HFD + Feiolix mid dose	796[c]	23.9	3.5[a]	2.7
HFD + Feiolix high dose	669[d]	22.9	-6.1[b]	2.7

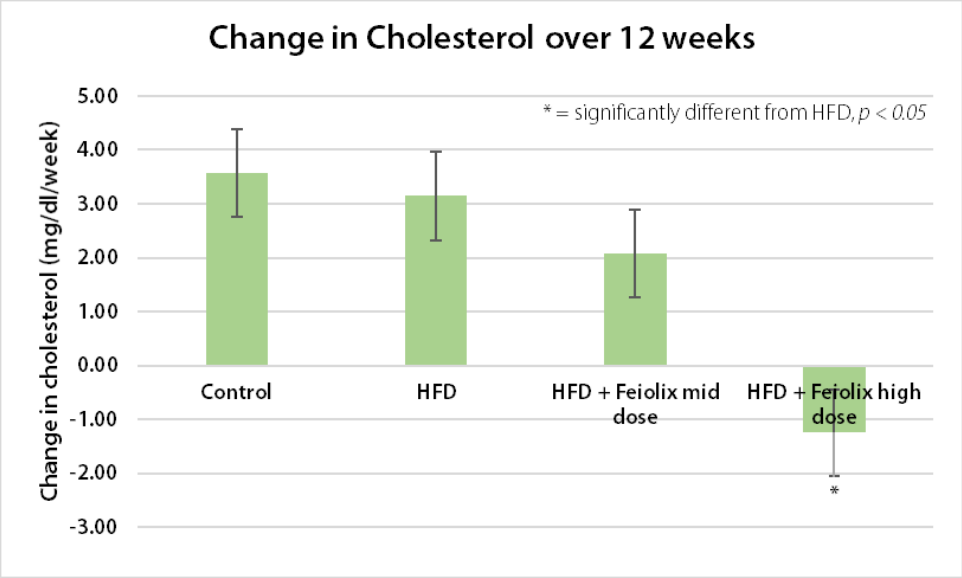




Cholesterol

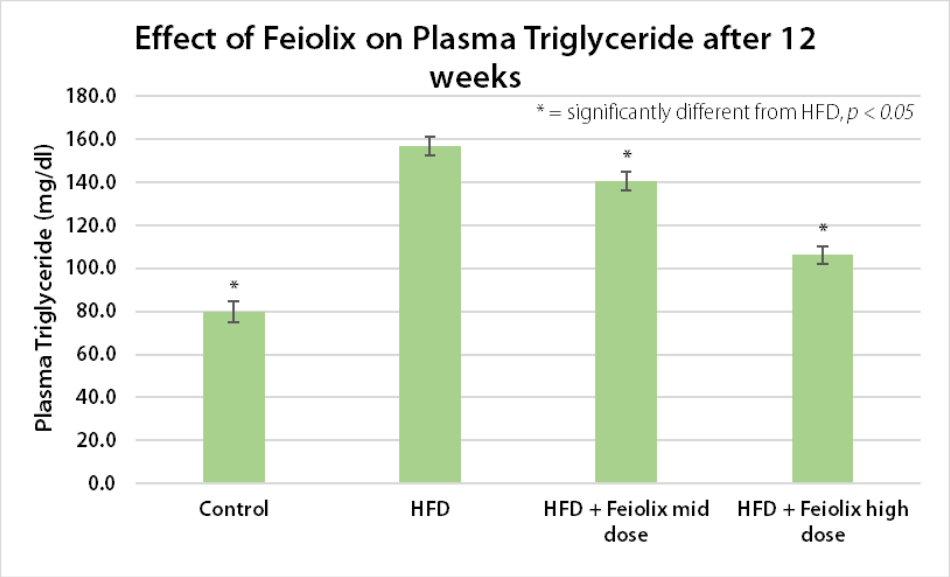
Treatment Group	Baseline Total Cholesterol at Week 20		Change in baseline Total Cholesterol of individuals (change as slope in per week (mg/dl) from W 8 to W 20)	
	Mean	SEM	Slope (mg/dl/week)	SE of the slope
Control	146.0[a]	7.92	3.58[a]	0.81
HFD	217.4[bc]	7.09	3.15[a]	0.82
HFD + Feiolix mid dose	203.3[c]	6.76	2.07[a]	0.81
HFD + Feiolix high dose	165.9[a]	6.47	-1.24[b]	0.80

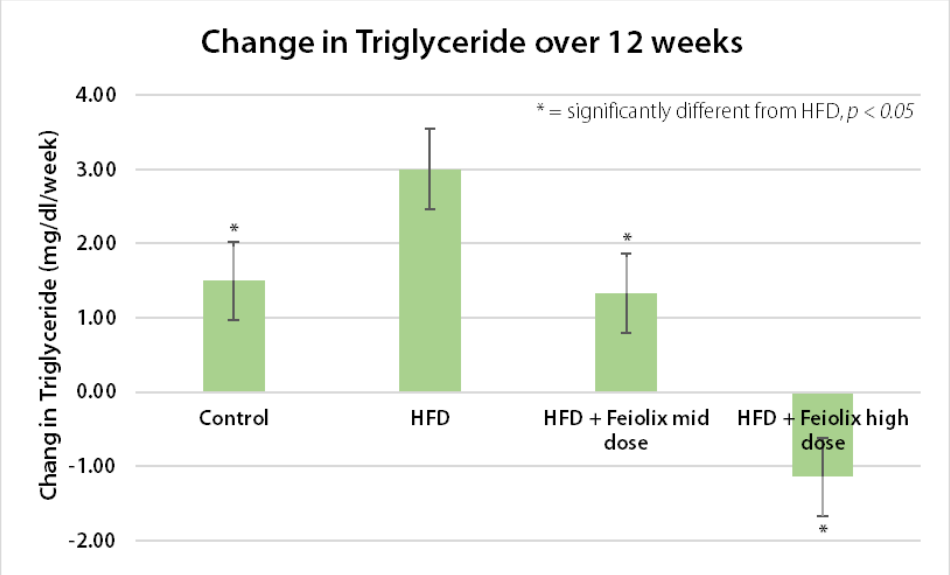




Tri-glyceride

Treatment Group	Baseline Tri-glycerides at Week 20		Change in baseline Tri-glycerides of individuals (change as slope per week (mg/dl) from W 8 to W 20)	
	Mean	SEM	Slope (mg/dl/week)	SE of the slope
Control	79.8[a]	4.93	1.50[ac]	0.53
HFD	156.9[b]	4.41	3.00[b]	0.54
HFD + Feiolix mid dose	140.5[c]	4.20	1.33[c]	0.53
HFD + Feiolix high dose	106.3[d]	4.02	-1.14[d]	0.53





1.3. THE EFFECT OF FEIJOA EXTRACT ON IMMUNE FUNCTION IN AGED MICE ANIMAL MODEL [PUBLISHED IN PATENT SPECIFICATION]

1.3.1. Summary Notes

- IL-2, IL-4, IL-6 and IFN are inflammatory cytokines important in the B-cell production of antibodies and are key components of the adaptive immune system.
- Aging is a major risk factor for the development of diabetes and its complications and thus reduced inflammatory cytokines also help lower the risk for diabetes.
- In this study, aged mice were divided into groups according to diet: a control group and a feijoa extract supplemented group.
- Mice in the feijoa group were fed a diet supplemented with feijoa fruit extract at a rate of approximately 1.8 mg/aged mouse/day for 32 weeks.
- Mice were sacrificed at the end of the 32-week period and levels of inflammatory cytokines were measured.
- Experimental groups:

Mice group		Body weight (g)	Spleen (mg)	Liver (g)	Heart (mg)
Mouse age	Treatment				
Young	Chow diet	26.36 ± 3.20	89.71 ± 16.25	1.46 ± 0.23	153.48 ± 14.12
Aged	Chow diet	43.08 ± 2.30	174.95 ± 15.15	1.76 ± 0.15	163.88 ± 16.81
Aged	Chow diet + feijoa	44.2 ± 3.1	133.5 ± 19.2	1.7 ± 0.2	160.2 ± 12.9

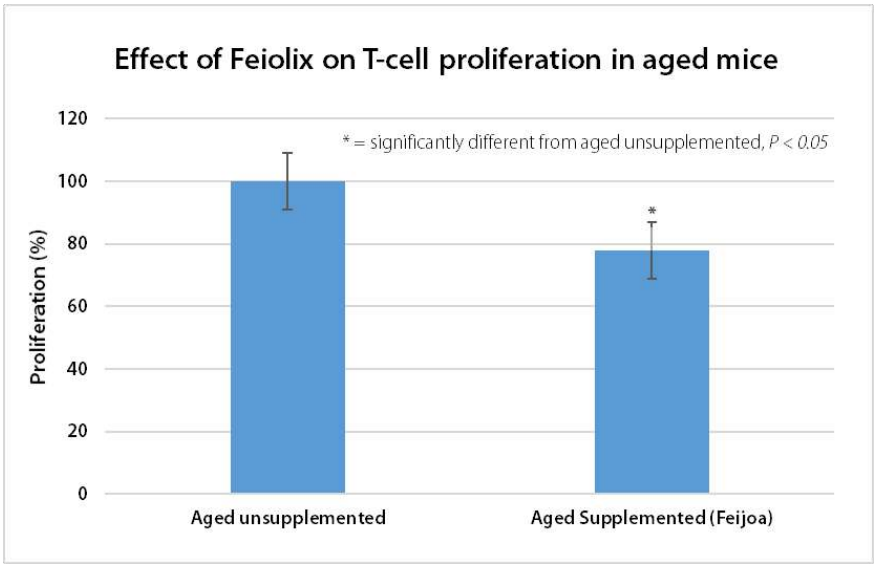
Mice supplemented with Feijoa fruit extract consume 6 g food/day, which equates to1.8 mg of extract/day. The feijoa fruit extract is added to the diet at a dose of 300mg/kg. Data indicates mean ± SD from 6 mice per group.

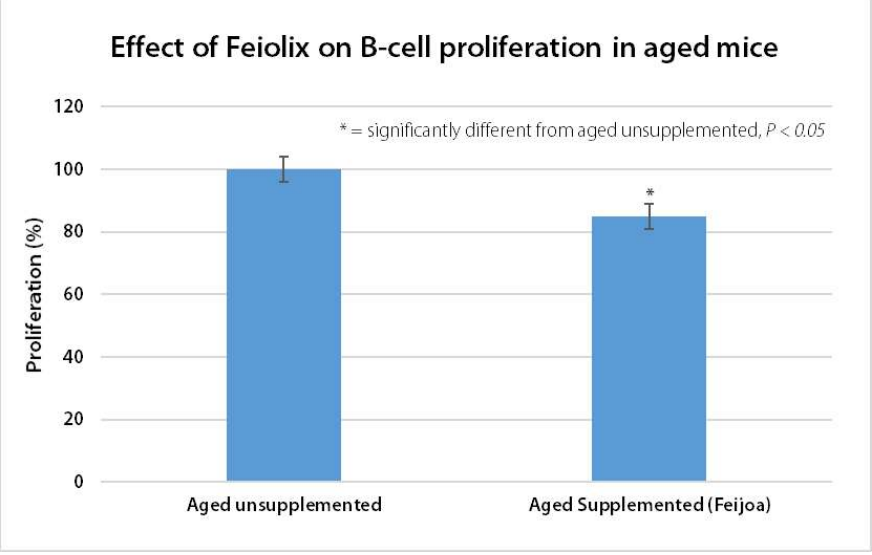
- Aged mice supplemented with feijoa extract significantly decreased splenic T-cell production of IL-4, TNF-α and TNF-β. IL-2 and IFN-γ production was not significantly affected in either groups.
- Aged mice supplemented with feijoa fruit extract at a dosage of 1.8 mg/day show a decrease in their IL-4 production by 44 % ($P < 0.01$) versus the aged un-supplemented group.
- Aged mice supplemented with feijoa fruit extract show a 20 % decrease in Concanavalin A stimulated splenic T-cell mitogenesis vs. aged mice not fed the feijoa fruit extract.
- Aged mice supplemented with feijoa fruit extract show a 14 % decrease in Lipopolysaccharide stimulated splenic B-cell mitogenesis.
- Aged animals often have spontaneously stimulated B–cells, which do not function as well as those in younger animals and also inhibit T-cells. Therefore, lowering mitogenesis or cell division by B-cells should be beneficial to host defences.
- Immunosenescence (deterioration of the immune system by age advancement) is a major contributing factor in survival to old age or premature death in humans and animals. Some of the adverse effects include dysregulated cell division of T- and B-lymphocytes upon stimulation by mitogens in vitro, or pathogens in vivo with altered cytokine production.
- In the present studies, B- and T-lymphocytes from aged mice divide less than those of young mice, and those from aged mice fed the feijoa fruit extract.
- The key observations on regulatory cytokines include stimulation of INF-γ, TNF-α, -β and -γ, and IL-4 by consequences of immunosenescence in aging.
- The lowering of these cytokines due to consumption of dietary feijoa fruit extract suggests better overall immune regulation, which can provide improved disease resistance.

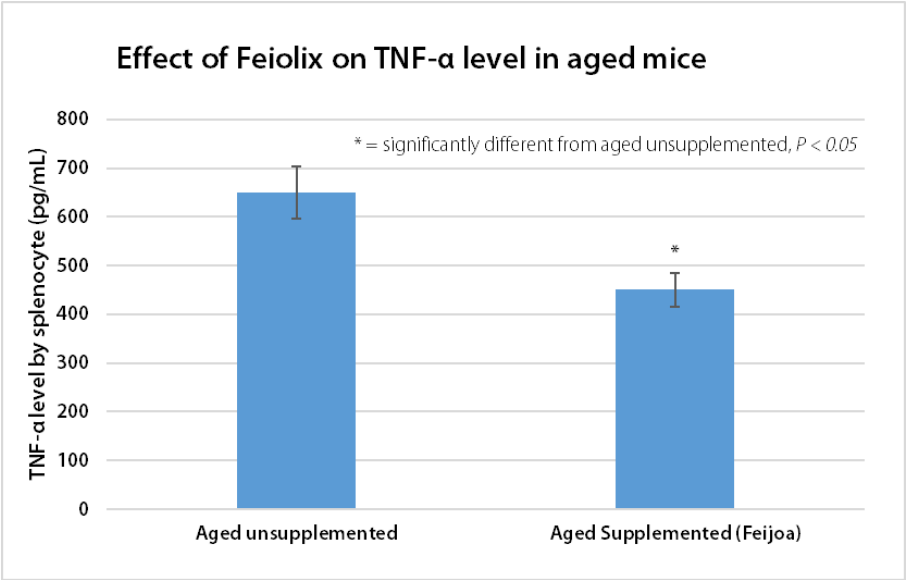
1.3.2. Results

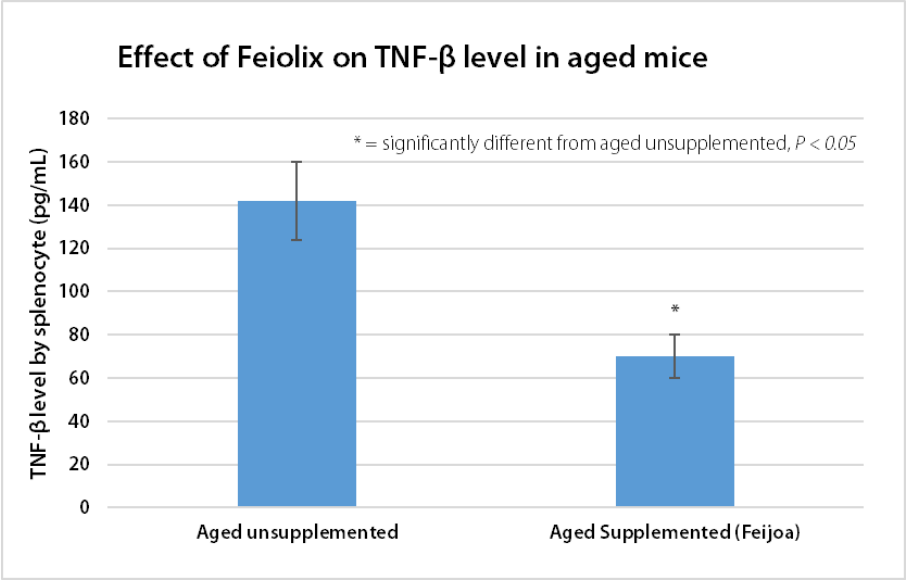
	Young Unsupplemented	Aged Unsupplemented	Aged Supplemented (Feijoa)
T-cell proliferation (%) (aged unsupplemented =100)	130 ± 15*	100 ± 9	78 ± 9*
B-cell proliferation (%) (aged unsupplemented =100)	130 ± 8*	100 ± 4	85 ± 4*
IL-2 level by splenocytes (pg/mL)	278 ± 26	264 ± 38	216 ± 47
IFN-γ level by splenocytes (pg/mL)	1555 ± 12	750 ± 37	907 ± 12
TNF-α level by splenocytes (pg/mL)	290 ± 30*	650 ± 53	450 ± 35*
TNF-β level by splenocytes (pg/mL)	95 ± 20*	142 ± 18	70 ± 10*
IL-4 level by splenocytes (pg/mL)	135 ± 40	165 ± 45	90 ± 30*
MDA levels in liver tissue (mol/mg protein)	0.12 ± 0.02*	0.325 ± 0.07	0.19 ± 0.03*
Hepatic vitamin E level (%) (aged unsupplemented =100)	200 ± 26*	100 ± 9	177 ± 3*

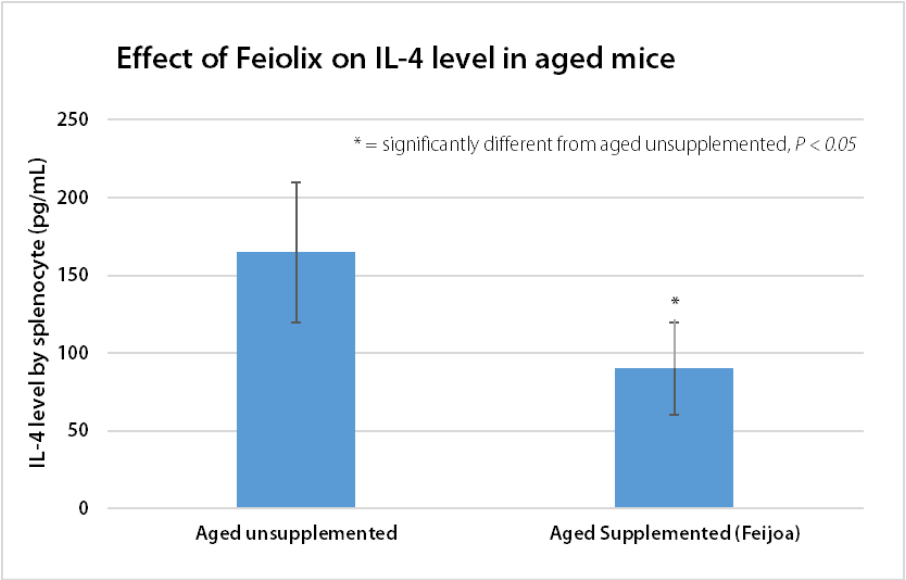
Data indicates mean ± SD from 6 mice per group. * Shows the statistical significance compared to aged control determined by unpaired Students t-test.

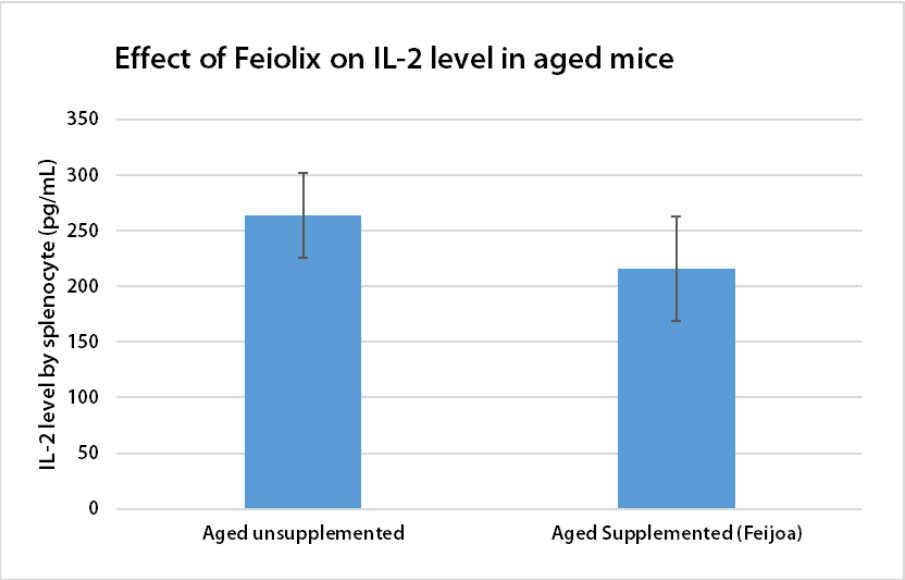


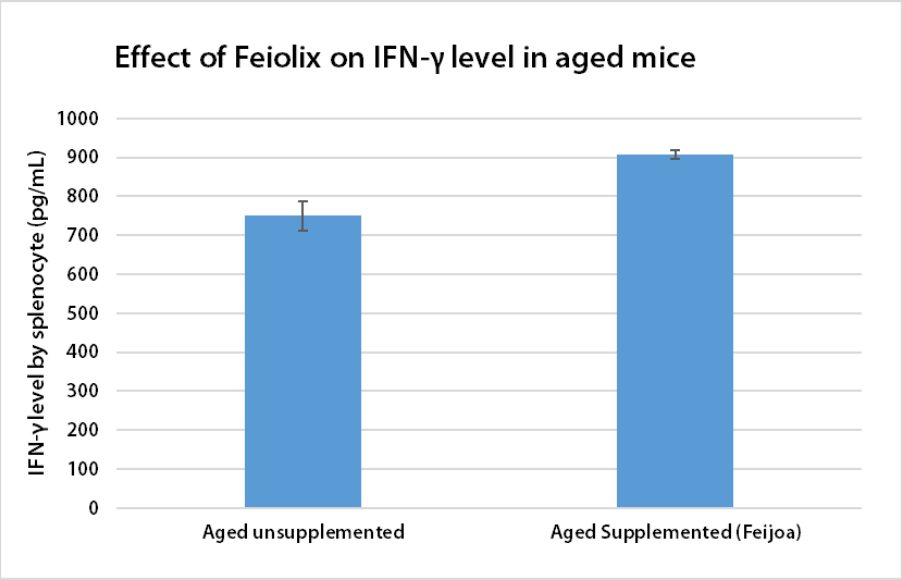



Effect of Feiolix on TNF-α level in aged mice
* = significantly different from aged unsupplemented, P < 0.05
TNF-α level by splenocyte (pg/mL)
800
700
600
500
400
300
200
100
0
*
Aged unsupplemented
Aged Supplemented (Feijoa)


Effect of Feiolix on TNF-β level in aged mice
* = significantly different from aged unsupplemented, P < 0.05
TNF-β level by splenocyte (pg/mL)
180
160
140
120
100
80
60
40
20
0
*
Aged unsupplemented
Aged Supplemented (Feijoa)


Effect of Feiolix on IL-4 level in aged mice
* = significantly different from aged unsupplemented, P < 0.05
IL-4 level by splenocyte (pg/mL)
250
200
150
100
50
0
*
Aged unsupplemented
Aged Supplemented (Feijoa)


Effect of Feiolix on IL-2 level in aged mice
IL-2 level by splenocyte (pg/mL)
350
300
250
200
150
100
50
0
Aged unsupplemented
Aged Supplemented (Feijoa)


Effect of Feiolix on IFN-γ level in aged mice
IFN-γ level by splenocyte (pg/mL)
1000
900
800
700
600
500
400
300
200
100
0
Aged unsupplemented
Aged Supplemented (Feijoa)

2. IN VITRO STUDY

2.1. EVALUATION OF THE ANTI-INFLAMMATORY PROPERTIES OF FEIJOA EXTRACTS [UNPUBLISHED]

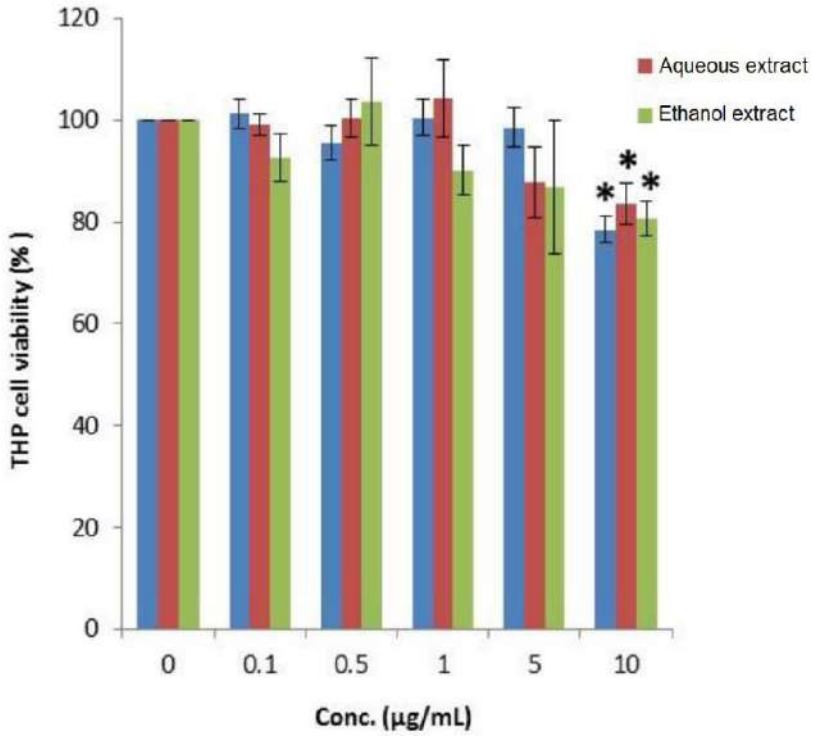
2.1.1. Summary Notes

- This study explored the potential ability of feijoa extracts to ameliorate inappropriate inflammation associated with chronic inflammatory disorders (e.g. metabolic syndrome/type-2 diabetes, arthritis).
- Chronic inflammatory disorders are primarily driven by "activated" immune cells (e.g. neutrophils, monocytes/macrophages).
- Two immune cell bioassays were used to assess the "anti-inflammatory properties" of the feijoa extracts:
  - Monocyte bioassay: Measures the expression of pro-inflammatory mediators, TNFα and IL-1β, in response to the bacterial ligand lipopolysaccharide (LPS) in THP-1 monocytic cell-line;
  - Neutrophil bioassay: Monitors changes in superoxide generation (i.e. oxidative burst) after phorbol 12-myristate 13-acetate (PMA) activation in peripheral neutrophils isolated from human blood donors.
- Feijoa extracts inhibited the secretion of LPS induced pro-inflammatory mediators, TNFα and IL-1β, from activated monocytes/macrophages.
- Feijoa extracts inhibited superoxide generation as well as scavenged/neutralised released superoxide from activated peripheral human neutrophils.
- Feijoa bioactives may be able to support foods for chronic inflammatory conditions.

2.1.2. Results

Evaluation of feijoa extract on cell viability (cytotoxicity)

- Feijoa extracts (aqueous and ethanol) did not have significant cytotoxic effects on THP-1 cell-line below 10 µg/ml concentration.



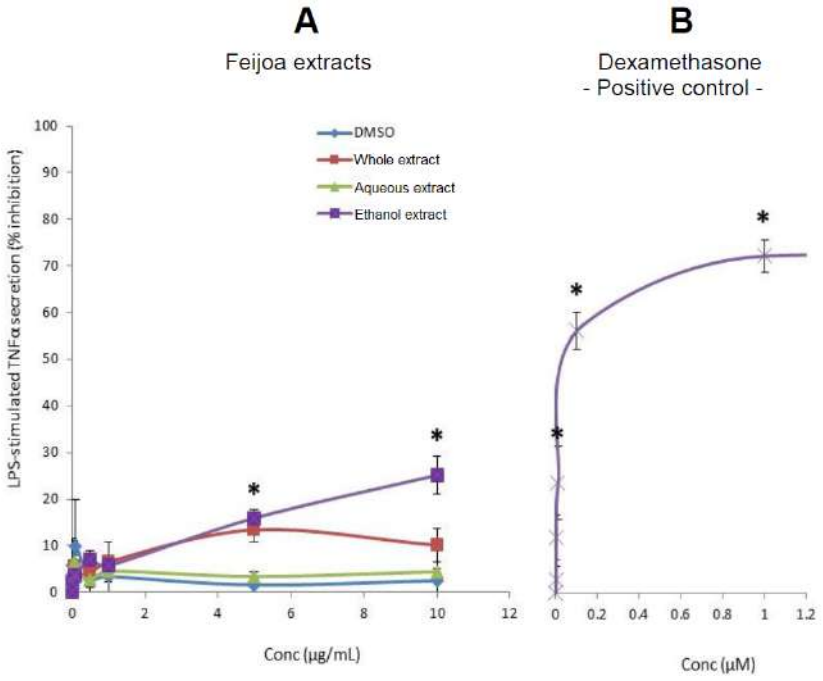
Feijoa extracts exhibit a dose-dependent cytotoxic action in a monocytic (THP-1) cell-line stimulated with lipopolysaccharide (5 ng/mL) after 6 hrs. Results are shown as % cell viability.

Values are mean ± SEM. (n=6 separate experiments).

* P<0.05 represents statistical significance from control cells.

Inhibition of LPS stimulated pro-inflammatory cytokine secretion

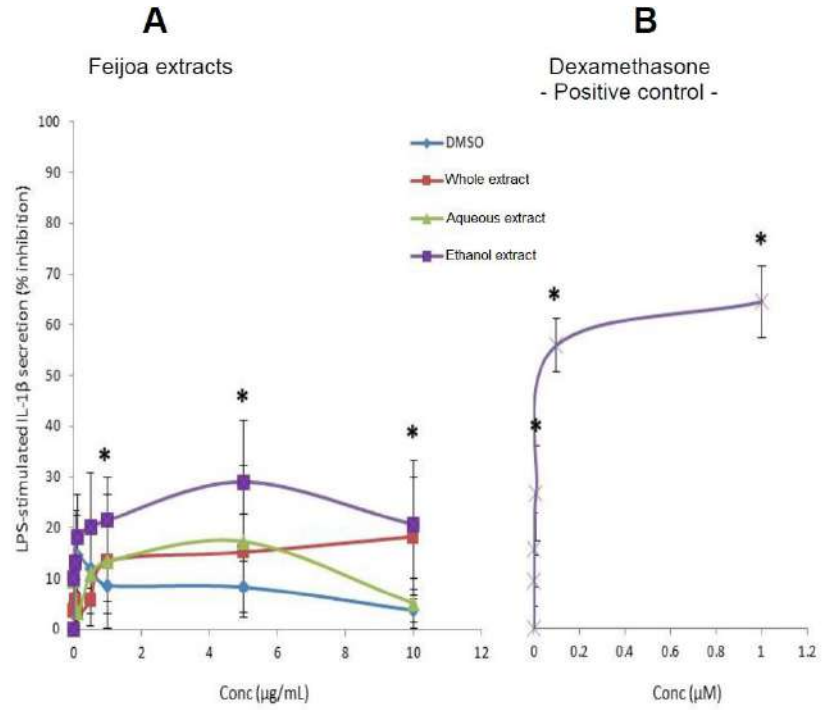
- Feijoa extracts inhibited LPS stimulated secretion of pro-inflammatory cytokine TNFα and IL-1β.



[A] Feijoa extracts and **[B]** dexamethasone exhibited a dose-dependent ability to inhibit LPS-stimulated tumour necrosis factor-α (TNFα) secretion from a monocytic (THP-1) cell-line after 6 hrs. Results are shown as % inhibition of LPS-stimulated TNFα secretion.

Values are mean ± SEM (n=6 separate experiments).

* P<0.05 represents statistical significance from LPS controls.



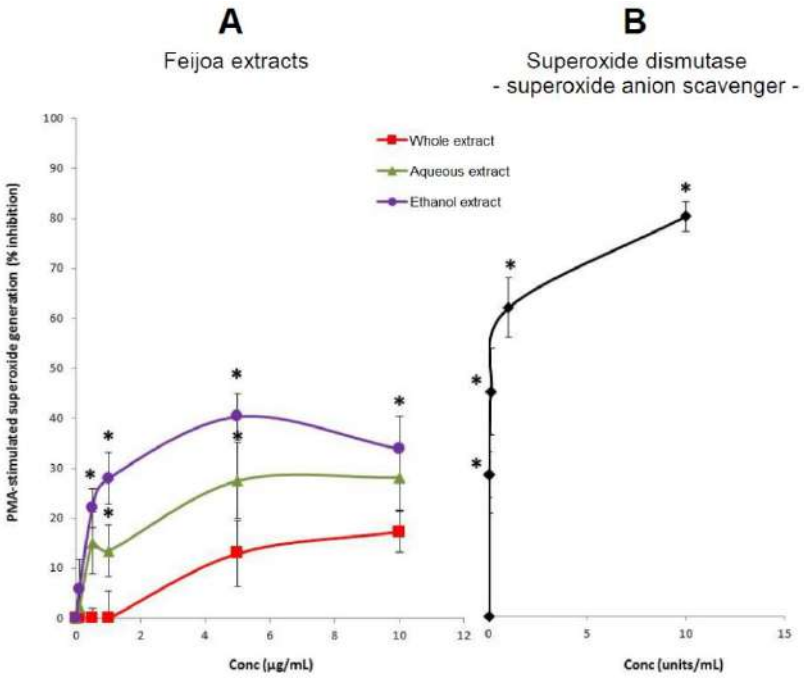
[A] Feijoa extracts and **[B]** dexamethasone exhibited a dose-dependent ability to inhibit LPS-stimulated interleukin-1β (IL-1β) secretion from a monocytic (THP-1) cell-line after 6 hrs. Results are shown as % inhibition of LPS-stimulated IL-1β secretion.

Values are mean ± SEM (n=6 separate experiments).

* P<0.05 represents statistical significance from LPS controls.

Prevention of neutrophil oxidative burst

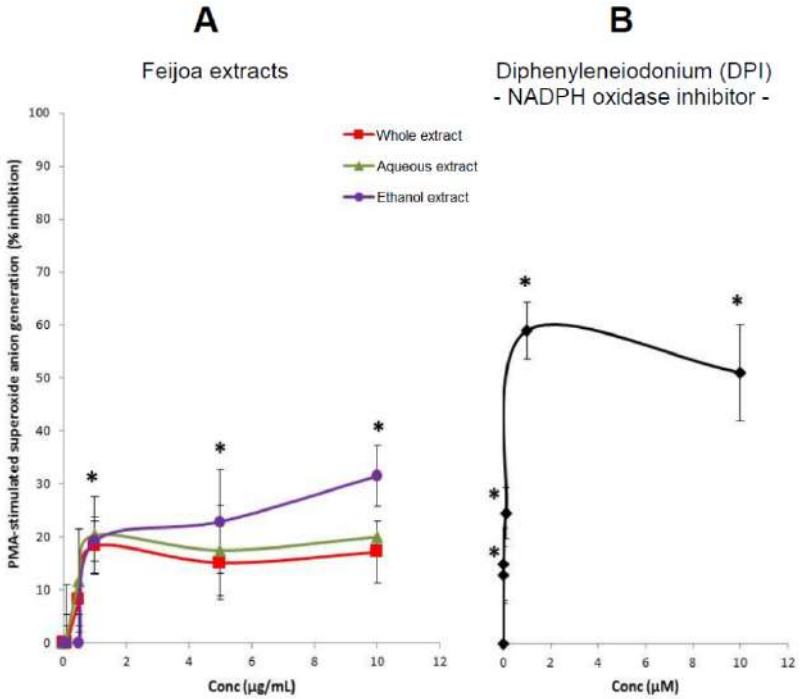
- Feijoa extracts scavenged superoxide released from PMA activated neutrophils.
- Feijoa extracts inhibited superoxide generation in PMA activated neutrophils.



[A] Feijoa extracts and **[B]** superoxide dismutase (SOD) exhibit a dose-dependent ability to scavenge PMA-stimulated superoxide released from peripheral neutrophils. Results are shown as % inhibition of superoxide released from PMA-stimulated neutrophils.

Values are mean ± SEM (n= experiments from four independent blood donors).

* P<0.05 represents statistical significance from PMA controls.



[A] Feijoa extracts and **[B]** Diphenyleneiodonium (DPI) exhibit a dose-dependent inhibition of PMA-stimulated superoxide generation in peripheral neutrophils. Results are shown as % inhibition of superoxide generation from PMA-stimulated neutrophils.

Values are mean ± SEM (n=experiments from four independent blood donors).

* P<0.05 represents statistical significance from PMA controls.