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The Effects of Rhein and Thymoquinone on Obesity and Diabetes in Diet-induced Obese Mice.

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The effects of Rhein and Thymoquinone on obesity and diabetes in diet-induced obese mice.

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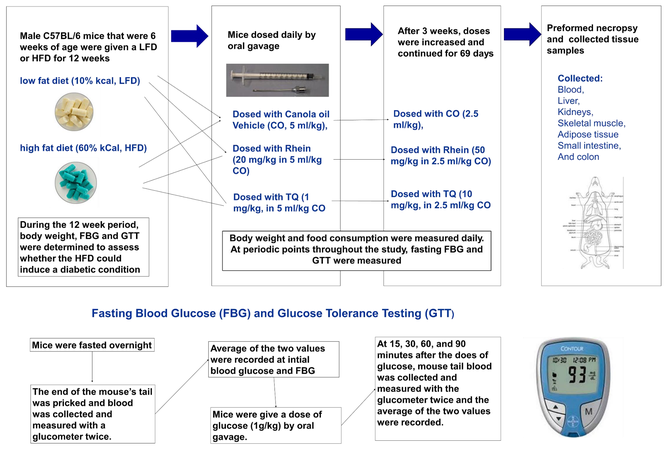
Introduction

Natural product extracts and chemicals isolated from natural products (e.g. plants, berries, seeds) have been commonly used in various types of traditional medicines. In addition, some drugs on the market today have been derived from natural product sources. The purpose of our study was to evaluate two natural products, Rhein and Thymoquinone, as potential anti-diabetic and anti–obesity agents. According to the Center for Disease Control (CDC), the number of people in the US diagnosed with diabetes has increase from 11.9 million people in the year 2000 to 20.8 million people in the year 2011. Rhein is a natural compound and a major component of *Rheum palmatum*, or Rhubarb. It has been used in Chinese medicine to treat constipation, gastrointestinal hemorrhage, ulcers as well as metabolic disorders such as diabetes. Recently, Rhein had been shown to improve non-alcoholic fatty liver disease (NAFLD) at does of 150 mg/Kg/day in diet induced obese mice through reducing body fat, improving serum lipid and glucose metabolism, and decreasing liver lipids, and reversing hepatic steatosis (Sheng et al., 2011). Rhein has also been shown to bind to and effect the Liver X receptors (LXRs) which play important roles in regulating cholesterol homeostasis, and lipid and energy metabolism. (Sheng et al., 2012). Thymoquinone (TQ) is a compound found in the plant *Nigella sativa,* or black cumin and has been documented to exhibit anti-diabetic, anti-obesity, hypotensive and hypo-lipidemic properties in human and animal studies. (Razavi and Hosseinzadeh, 2014). Extracts from *Nigella sativa* significantly increased hepatic and intestinal apolipoprotein A-I which is a major protein component of high density lipoprotein (HDL) secretion. The extract also induced peroxisome proliferator-activated receptor alpha (PPARα) expression by 9-fold and retinoid X receptor alpha (RXRα) expression by 2.5-fold. (Haas et al., 2014). The PPARα and RXRα nuclear receptors play and important role in regulating major lipid metabolism proteins. The purpose of our study was to evaluate whether daily administration of Rhein or TQ could improve obesity-induced diabetes in mice. First, male C57BL/6 mice that were 6 weeks of age were fed a low fat diet (10% kcal, LFD) or a high fat diet (60% kCal, HFD) for 12 weeks. Over the twelve-week period, body weight, fasting blood glucose and glucose tolerance were determined to assess whether the high fat diet could induce a diabetic condition. Starting from week 12, mice were administered canola oil vehicle (CO, 5 ml/kg), Rhein (20 mg/kg, 5 ml/kg in CO), or TQ (1 mg/kg, 5 ml/kg) daily. After three weeks of dosing the Rhein and TQ doses were increased to Rhein (50 mg/kg, 2.5 ml/kg) and TQ (10 mg/kg, 2.5 ml/kg). There were six groups of mice in this study, with the following groups: i) LFD + CO, ii) LFD + Rhein, iii) LFD + TQ, iv) HFD + CO, v) HFD + Rhein, vi) HFD + TQ. Body weight and food consumption were measured daily. At periodic points throughout the study, fasting blood glucose (FBG) and glucose tolerance (GTT) were measured in the mice.

Materials and Methods



Analysis

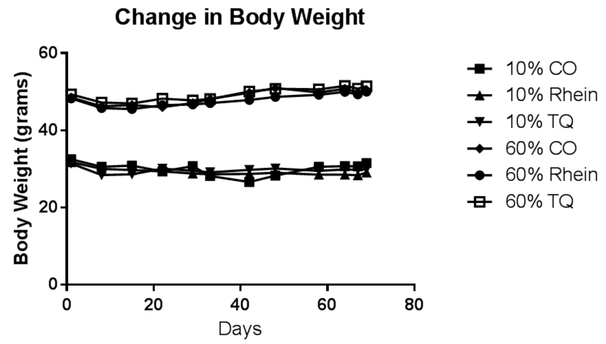


Figure 1. Changes in average body weights in grams throughout the 69 days of dosing within the treatment groups.

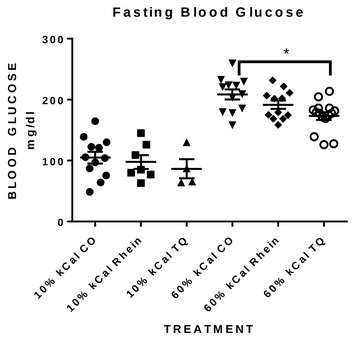


Figure 2. Fasting blood glucose (mg/dL) of different treatment groups measured at day 23 of dosing.

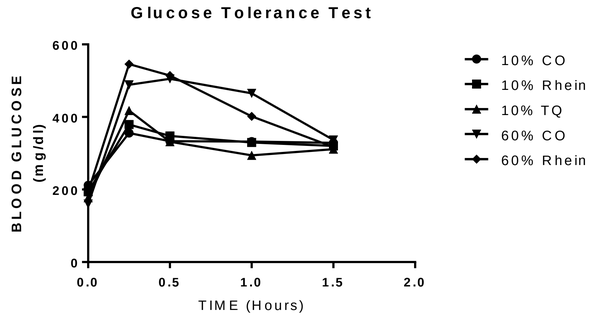


Figure 3. GTT done at day 56 of dosing for the treatment groups (Missing 60% TQ group).
Average blood glucose measurements in (mg/dL) taken at various time points, after giving a oral dose of glucose (1 mg/kg).

Conclusions

- There are differences in body weight, FBG, and GTT between the mice feed a HFD and LFD as expected
- The body weights, FBG, and GTT of the groups dosed with rhein compared with the groups dosed with the vehicle CO show little differences
- The body weights and GTT of the groups dosed with TQ compared with the groups dosed with the vehicle CO show little differences
- There is a slight difference between the FBG of the HFD group dosed with TQ compared with the HFD group dosed with the vehicle CO. Which may be an indication that TQ may lower blood sugar but more studies need to be conducted and this group is also missing from the GTT data due to unforeseen circumstances

Future Studies

- Blood, liver, kidneys, skeletal muscle, adipose tissue, small intestine, and colon tissues were collected a further analysis should be conducted in these tissues such as a Glycated hemoglobin (A1c or HbA1c) measurement from the blood tissue which would identify the average plasma glucose concentration over a prolonged period of time.
- Further analysis of the tissues after homogenization, which will break apart cells so protein expression can be analyzed would be helpful to determine if the natural products had an effect on mechanisms of obesity and diabetes.
  - Western blotting of LXR, PPARα, PPARγ and RXR would be useful to see if Rhein or TQ had an effect on these nuclear receptor that control energy metabolism.
  - Western blotting of IRS-1, GLUT2, GLUT4 and SREBP-1c would be useful to see other mechanisms of glucose and lipid regulation by transporters and transcription receptors.

References

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CDC - Number of Adults - Diagnosed Diabetes - Data & Trends - Diabetes DDT.

Poster was presented at 2015 Honors Undergraduate Conference, Kingston, RI

Male C57BL/6 mice that were 6 weeks of age were given a LFD or HFD for 12 weeks

low fat diet (10% kcal, LFD)

high fat diet (60% kCal, HFD)

During the 12 week period, body weight, FBG and GTT were determined to assess whether the HFD could induce a diabetic condition

Mice dosed daily by oral gavage

Dosed with Canola oil Vehicle (CO, 5 ml/kg),

Dosed with Rhein (20 mg/kg in 5 ml/kg CO)

Dosed with TQ (1 mg/kg, in 5 ml/kg CO

After 3 weeks, doses were increased and continued for 69 days

Dosed with CO (2.5 ml/kg),

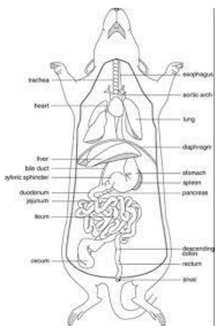
Dosed with Rhein (50 mg/kg in 2.5 ml/kg CO)

Dosed with TQ (10 mg/kg, in 2.5 ml/kg CO

Body weight and food consumption were measured daily. At periodic points throughout the study, fasting FBG and GTT were measured

Preformed necropsy and collected tissue samples

Collected:
Blood,
Liver,
Kidneys,
Skeletal muscle,
Adipose tissue
Small intestine,
And colon



Fasting Blood Glucose (FBG) and Glucose Tolerance Testing (GTT)

Mice were fasted overnight

The end of the mouse's tail was pricked and blood was collected and measured with a glucometer twice.

Average of the two values were recorded at initial blood glucose and FBG

Mice were give a dose of glucose (1g/kg) by oral gavage.

At 15, 30, 60, and 90 minutes after the does of glucose, mouse tail blood was collected and measured with the glucometer twice and the average of the two values were recorded.