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Efficacy of *Hoodia gordonii* Extract as a Weight Loss Supplement: A Comparative Study Between an Invertebrate, *Tenebrio molitor* (Coleoptera: Tenebrionidae), and a Vertebrate, *Rattus norvegicus* (Rodentia: Muridae)

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ABSTRACT

Despite legal constraints, *Hoodia gordonii* is marketed in many over-the-counter forms claiming to promote weight loss by reducing appetite. The belief that this plant is an efficacious appetite suppressant is based in traditional knowledge from the San tribes of the Namibian desert. We tested the efficacy of one commercial form of this plant extract to reduce feeding behavior, and therefore promote weight loss, in two different organisms. We used Sprague-Dawley rats, *Rattus norvegicus*, as a human analog to predict the usefulness of this plant as a dietary supplement for humans. We also used the adult mealworm beetle, *Tenebrio molitor,* to compare an invertebrate species with the vertebrate results. *T. molitor* is a close relative of many beetle species native to the same region in which *H. gordonii* naturally grows. The control group contained organisms not exposed to *H. gordonii*. The experimental group received a body mass equivalent dosage of *H. gordonii* in solution with distilled water for a month. We monitored food consumption and body weight for one month to compare the control group results with those of the organisms that ingested *H. gordonii* solution. This study does not provide support that the commercial plant product used is efficacious as a hunger suppressing dietary supplement in either species.

INTRODUCTION

Hoodia gordonii is a desert grown plant native to South Africa and the Namib Desert that has been utilized for generations by the San people of South Africa for its supposed ability to suppress hunger. This plant has recently been exploited and processed into a highly popular diet strategy marketed and sold in a variety of forms through multiple providers (Consumer Reports on Health, 2006). In 2003, an economical and legally binding arrangement was reached between the San tribes and the Council for Scientific and Industrial Research (CSIR) of South Africa, which allows these parties to monitor and control the production and sale of any products containing *H. gordonii* in an effort to pre-

vent over-exploitation of this resource (WHO, 2006). This treaty also gave the San people rights to a portion of the profit and royalties associated with the production and sale of *H. gordonii*-containing products (Vermeylen, 2007). With an emphasis placed on the maintenance of sufficient population levels, strict regulations have been placed on the harvesting and trade of this plant for commercial use. This limitation has often led suppliers to dilute the concentration of extract within the marketed forms (Vermaak et al., 2010).

Despite the popularity of this plant extract, little scientific data have been collected with regards to the actual effects of this plant compound. Larvae of the cabbage looper moth, *Trichoplusia ni*, have been used to determine the carry-over of larval *H. gordonii*-containing diet to the adult stage after pupation (Chow et al., 2005; Shikano and Isman 2009). Not only was this species studied to determine the effects of ingested *H. gordonii*, but also to determine the effects of topical application of the plant extract to the larval, pupal, and adult stages of *T. ni* (Akhtar et al., 2009). Ingestion of *H. gordonii* caused a significant change in oviposition site preference; topical application of *H. gordonii* had no effect on this behavior. Rader et al. (2007) determined the chemical composition of the plant extract, mainly as a method of determining the purity of marketed forms, and identified P57, an oxypregnane steroidal glycoside found in many weight loss supplements, as one of the primary components in *H. gordonii*.

To study the efficacy of *H. gordonii* on a close relative of the native South African beetle species, we chose to study the adult mealworm beetle, *Tenebrio molitor*. Mealworm beetles, family Tenebrionidae, are commonly referred to as darkling beetles. By testing the effects of *H. gordonii* extract on the feeding behavior of a close relative to the native species of tenebrionid beetle, we hope to develop results that can be compared to the species of beetle that would potentially feed on this plant in the wild (Hamilton et al., 2003). If *H. gordonii* does act as a hunger suppressant, and the tenebrionid beetles naturally exposed to this plant ingest its material, the overall food intake over the lifetime of an individual beetle may be significantly reduced. This reduction in caloric intake, and thereby reduction in energy gain, may result in decreased reproductive success of those that consume the plant. The small size of this beetle makes it an ideal organism to study because it is cost and space efficient. This efficiency allows us to increase the sample size of our test groups, allowing the study to more accurately represent the variability present in natural settings.

We also studied the efficacy of *H. gordonii* on Sprague-Dawley rats, *Rattus norvegicus*, because of the common usage of this organism as a human analog species for pre-clinical research (Boozer and Herron, 2006). It is important to test the efficacy of pharmaceutical candidates on a human analog prior to performing clinical research, and the Sprague-Dawley rat is historically a popular choice for this type of research (Fallon et al., 2008). *R. norvegicus* is a relatively accessible and affordable species making it a popular choice as a research organism. Not only does research on rats allow us to predict the efficacy of *H. gordonii* as a weight loss supplement for humans, but it also allows us to conduct a comparative study on two organisms that are not closely related. This comparative analysis should provide information about any possible differences in the efficacy of *H. gordonii* as a hunger suppressant in invertebrates versus vertebrates. Although the size of these

rats limits the sample size, it also increases the accuracy of our techniques for administration of *H. gordonii* solution.

Our hypothesis, based on the popularity of commercialization and traditional use by the San tribes of South Africa, is that ingestion of *H. gordonii* will suppress the appetite of both *T. molitor* and *R. norvegicus*. This suppressing effect should be represented by a decrease in average daily food consumption over the trial period and subsequent weight loss in the organisms whose diet has been supplemented with *H. gordonii* solution at the end of the trial period.

MATERIALS AND METHODS

We conducted this research in the Leighty-Tabor Science Center at Millikin University in Decatur, Illinois. The control group received no plant extract solution and the experimental group received a body mass equivalent dosage of *H. gordonii* extract in solution with distilled water. Although the concentrations of the solution used for each trial differed based on the average starting weight of the animals, we prepared these solutions using the same *H. gordonii* extract, in capsule form, purchased from Mari-Mann Herb Farm in Decatur, Illinois.

We separated the beetles and rats into control and experimental groups based on relative body size by alternately placing the lightest individual of each species into a control group and the next lightest into an experimental group. This separation method ensured that the weight distribution between the two groups was almost equivalent, thereby eliminating body size as a variable on feeding rates. Although the measurement technique used for each organism differed because of the size of each organism used, we collected the same data for each trial. In order to test the efficacy of *H. gordonii* as an appetite suppressant, we measured the mass of food consumed. We then calculated the mean of these measurements to represent the average daily food consumption for each organism. We also recorded the body weight of each animal for the duration of each trial. At the end of the trials, we calculated the average final mass of each group of organisms and used this measurement to determine if the *H. gordonii* solution had caused a difference in weight. Since the average initial weights of each group were nearly equivalent, we were able to use only the final body mass to determine the efficacy of *H. gordonii* as a weight loss dietary supplement.

Due to the inconsistency present in the dietary supplement market, future research could address a chemical analysis of the *H. gordonii* extract used in this experiment. Since P57 has been identified as the active ingredient in *H. gordonii*, it would be useful determine the amount of compound, if any, present in the commercial extract used. High-performance thin layer chromatography (HPTLC) can be used to determine the presence of P57 (Vermaak et al., 2010). We used this method of biochemical analysis to determine that P57, the suspected active ingredient of *H. gordonii*, is present within the extract used in this research (Fig. 1). High-performance liquid chromatography (HPLC) can also be used to form a chemical fingerprint of the plant extract for comparison to the known chemical composition of pure *H. gordonii* (Avula et al., 2007). We are currently pursuing further methods of sample authentication.

***T. molitor* protocol**

We separated 100 male and female adult *T. molitor* beetles into two groups, control (n = 50) and experimental (n = 50). We supplied control beetles with distilled water and experimental beetles with a constant dose of *H. gordonii* plant extract in solution with distilled water. We prepared water dishes by taping the bottoms of two 60.0 mm polystyrene petri dishes together to form a sealed container, which we filled with two cotton balls prior to sealing. We then drilled a hole in the side of this container just big enough for the beetles to fit their heads inside to access the water supply. These containers were constructed for the purpose of controlling levels of evaporation; preliminary test results confirmed that this container design limited evaporation to an insignificant level.

We used the average weight of an adult human, 70 kg, to determine the dosage of *H. gordonii* per body weight ratio. We used this ratio to determine the daily dosage of *H. gordonii* extract to administer to the beetles based on the average mass of the beetles. We determined the total dosage for the entire group of beetles for the length of the trials and created a 1.0 L stock solution containing 0.024 g of powdered *H. gordonii* extract. Once we created this stock, we used a glass pipette to fill each water container in the experimental group with 1.0 mL of this solution. We filled each container in the control group with 1.0 mL of distilled water. We prepared the food for the beetles by mixing equal weights of wheat flour and whole oatmeal.

We individually housed each beetle in a 475 mL plastic container with approximately 100 mL of the prepared food mixture and covered the container with a ventilated plastic lid. We maintained each beetle in the same container for the duration of the experiment to ensure that any changes in the weights of the food were attributed to the appropriate beetle. To weigh the beetles, we removed them from their housing containers, one at a time, made sure they were free of any food particles or other debris, then placed them on their dorsal side in a plastic petri dish on the electronic balance. While the water containers were removed, and the lid was off, we weighed the entire food container. Prior to the trials, we tested the excrement production rates of the beetles by housing an individual beetle in an empty container for 24 hours. We found that, due to evaporation of waste, this production rate was insignificant when compared to the mass of the container. Therefore, we were able to attribute any changes in the mass of the container to consumption by the beetle being housed in that container. We tracked the weight of each container throughout the trial and calculated any change in the mass of the container that had occurred at each measurement period. We repeated this measurement process three times a week throughout the trial period of one month, recording each of the values to the nearest 0.001 g. We dispatched all remaining beetles by feeding them to a pet bearded dragon.

***R. norvegicus* protocol**

We separated 22 juvenile male *R. norvegicus* into two separate groups, control (n = 11) and experimental (n = 11). We gavaged the control rats three times a week with distilled water and the experimental rats three times a week with *H. gordonii* solution (see description below and in Talpur et al., 2001). We housed rats in individual plastic containers with base dimensions of 30 x 50 x 30 cm. These containers had grated stainless steel lids, equipped with food and water dispensing mechanisms, which allowed appropriate air flow and resource accessibility for the rats. We supplied the rats with *ad libitum* rat chow pellets. We labeled each housing container using a number between 1

and 22 and either an ‘A’ or a ‘B’. The rats in the group labeled with an ‘A’ were in the control group and those with a ‘B’ were in the experimental group. In order to measure the weights of the rats throughout the trial, we placed a foam bucket onto a balance to restrain the rats, eliminating the need for anesthesia, without affecting the accuracy of the measurements. The same balance was used to measure food consumption by measuring the mass of the remaining food at each measurement period and calculating the difference from the previous measurement. We weighed rats and food three times a week and recorded data to the nearest 0.01 g. At the conclusion of the trial, we dispatched all rats using an approved method of carbon dioxide euthanasia and post-euthanasia freezing.

The gavaging process is a modification of the protocol used by Talpur et al. (2001) who tested the efficacy of a mixture of Chinese herbal supplements as a weight loss supplement. We used the average weight of an adult human, 70 kg, to determine the *H. gordonii* dosage per body weight ratio. We used this ratio to determine the proper dosage to be administered to the rats in the experimental group based on their average initial body mass. We determined that the appropriate daily dosage, compared to 400 mg in humans, should be 12 mg per day. This dosage calculation allowed us to prepare a stock *H. gordonii* solution that contained 30 mg of *H. gordonii* plant extract per 1 mL of distilled water. This mixture, administered via a 5.08 cm curved stainless feeding needle three times a week, ensured an average dosage of 12 mg of *H. gordonii* per day per rat for the one month trial period.

Statistical Analysis

We calculated the mean weights for each of the groups, control and experimental, after each day of measurement, and then compared the means between the two groups using standard deviation calculations. The standard deviation of a data set is the root mean of the variance within the data set. Standard deviation utilizes the square root of the average of the deviation of each measurement from the mean; a small standard deviation indicates a data set in which most values fall close to the mean, while a large standard deviation indicates data that are spread out across the range. The standard deviation is used to create error bars when plotting the mean values between the two groups. If the error bars for each mean overlap one another, then there is no significant difference between the control and experimental groups. Additionally, we used a two-tailed t-test to determine the probability that any overlap was caused by random chance alone. This test determines a *P*-value, which is used to determine significance of any differences that may be present within the data while assuming unequal variance between the populations. A *P*-value greater that 0.05 indicates no significant difference in the data.

RESULTS

We omitted data collected for beetles that died before the end of the trials, leaving data for a total of 7 trial sets for 73 of the initial 100 beetles, which were able to be analyzed and compared between the two groups. The number of beetles remained close between the two groups throughout the experiment, with a total of 13 control beetles and 14 experimental beetles dying. There were no deaths in either rat groups. Both groups of *T. molitor* started with a mean body mass of 0.11 g. Both groups of *R. norvegicus* started with an initial mean body mass of approximately 210 g. This similarity in initial body mass between the control and experimental groups allowed us to use the mean final mass

to compare any change in body mass that might be due to ingestion of *H. gordonii* extract. There were no significant differences in either average final body mass or mean daily food consumption for either species. However, the average final body mass (Fig. 2) and average daily food consumption (Fig. 3) were slightly lower in the experimental group for the beetles. Similarly, the average final body mass (Fig. 4) and average daily food consumption (Fig. 5) were slightly lower in the experimental group for the rats.

DISCUSSION

Our hypothesis, that ingestion of *H. gordonii* plant extract would decrease food consumption resulting in a decrease in total body weight, was not supported. Although there was a trend toward decreased average daily food consumption in the experimental groups, as well as a trend toward decreased final body weight in these groups, these trends do not represent significant differences in the data. Therefore *H. gordonii* extract, when administered in a body mass equivalent dosage, does not seem to cause any significant change in feeding behavior or body weight in adult mealworm beetles or Sprague-Dawley rats.

Although *H. gordonii* extract did not produce significant differences in the feeding rates of *T. molitor* or *R. norvegicus*, it is still important to compare the results between the invertebrate and vertebrate subjects. In *T. molitor*, the difference between average daily food consumption by the experimental and control groups is larger than the difference in average final body mass of the two groups. In *R. norvegicus*, the difference between average daily food consumption by the experimental and control groups is smaller than the difference in average final body mass of the two groups. Although this variation is not represented by statistically significant differences, it does shed light on a potential difference in the type of effect, if any, which may be caused by ingestion of *H. gordonii*. This variation indicates that the invertebrate species might be exhibiting a behavioral response to the plant extract while the vertebrate species might be exhibiting a physiological response. The variation could also be attributed to differences in the metabolism of the two species. Although *H. gordonii* ingestion did not cause significant decrease in the feeding behavior of mealworm beetles, it did affect the oviposition preference of the cabbage looper moth, *Trichoplusia ni* (Chow et al., 2005; Shikano and Isman, 2009). Ingestion of *H. gordonii* by larval moths increased ingestion of *H. gordonii* in the adult moths. This indicates that *H. gordonii* is capable of producing an effect in an invertebrate species. Repeating our protocol on *T. ni* should allow us to further determine the efficacy of *H. gordonii* as an appetite suppressant in invertebrates.

We have anecdotal evidence that some of the rats, in both the control and experimental groups, exhibited behavioral changes throughout the duration of the trial. Some of the rats showed increased avoidance behavior and others showed increased aggression. Additionally, following gavaging, several rats in both groups moved quickly and randomly about the cage. Since we only have anecdotal evidence of the occurrence of these behavioral changes we cannot be sure of their cause. We can only speculate that they may be a result of associative learning.

We recognized two potential sources of error in the beetle protocol. It is possible that the large ventilation holes in the lid of the beetles' housing containers could have affected the overall mass of the containers by allowing moisture to move freely in and out of the con-

tainer. Additionally, although the electronic balance used is designed for extreme precision, other individuals were also using the same balance which could have resulted in calibration errors.

Although this research does not provide information about possible long-term effects of *H. gordonii* extract, it does provide some insight to the short-term effects of this plant on *T. molitor* and *R. norvegicus*. Based on our results, members of family Tenebrionidae that are naturally exposed to *H. gordonii* are not likely to be affected by ingestion of this plant material. Additionally, based on the lack of efficacy of this plant extract as a weight loss supplement in rats, it is likely that *H. gordonii* would not have a hunger suppressing effect on humans. It would be interesting to test this plant extract on humans to determine whether the San people were accurate in their predictions, but for the adult mealworm beetle and Sprague-Dawley rats, *H. gordonii* does not cause a significant decrease in body weight or feeding behavior.

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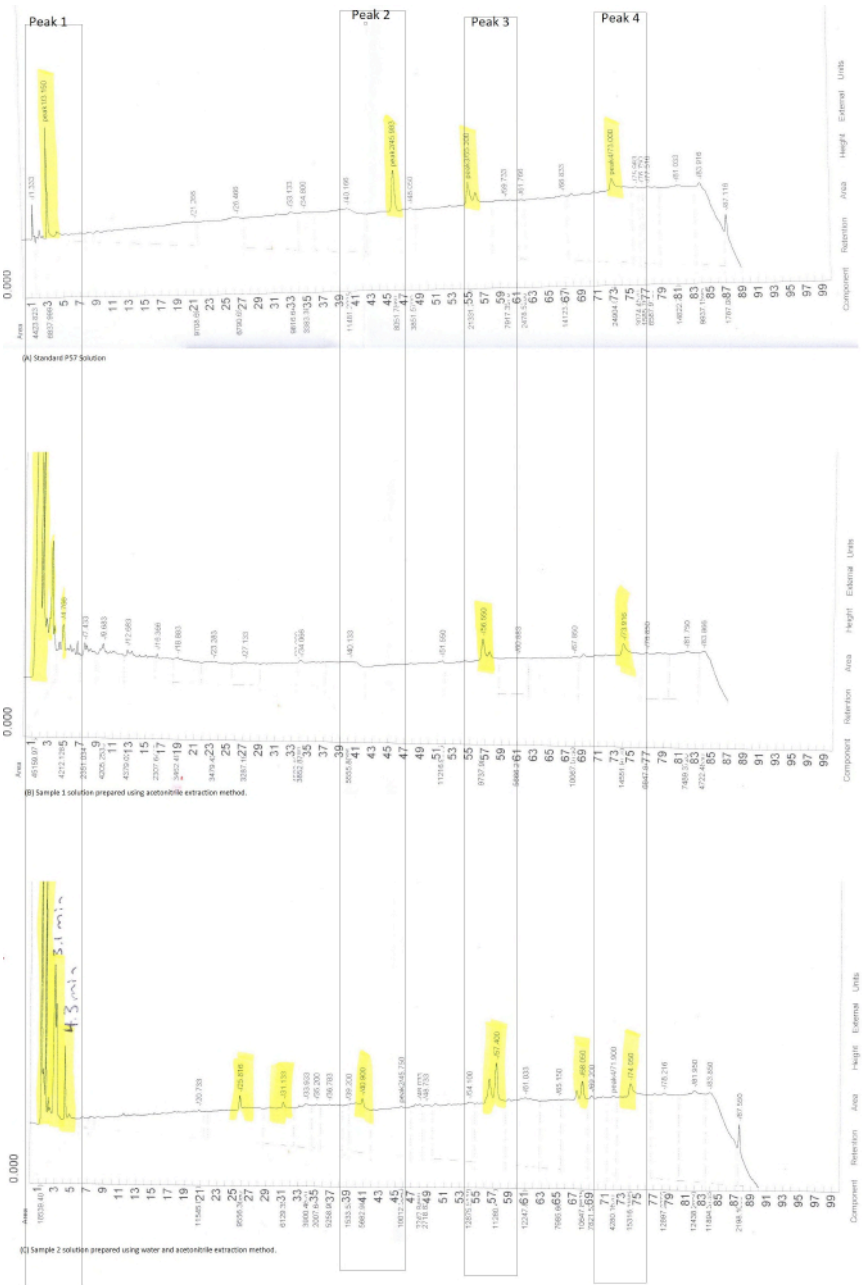

Figure 1. Comparison of HPLC profiles of P57 standard solution (A), *H. gordonii* extracted with acetonitrile (b), and *H. gordonii* extracted with acetonitrile and water (50:50), at a detection wavelength of 220 nm.

Figure 2. Comparison of average final body mass (± SD) of adult *Tenebrio molitor* beetles in the control group receiving distilled water versus an experimental group receiving 0.024g of *Hoodia gordonii*/L of distilled water *(P* = 0.141).

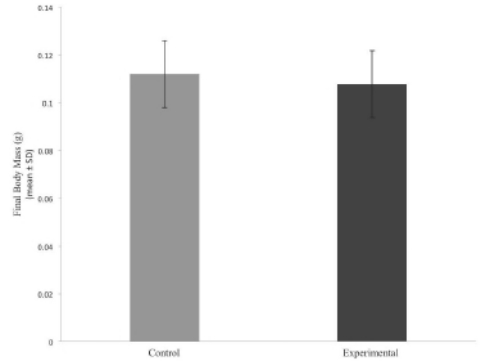

Figure 3. Comparison of mean daily food consumption by control adult *Tenebrio molitor* receiving distilled water versus an experimental group receiving 0.024g of *Hoodia gordonii*/L of distilled water *(P* = 0.405).

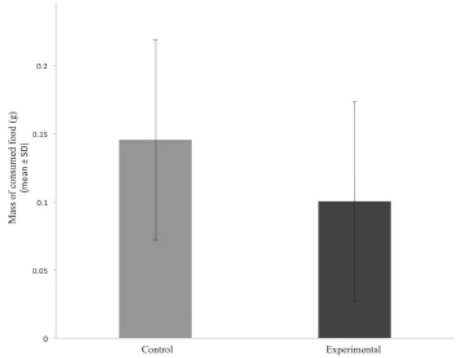

Figure 4. Comparison of average final body mass (± SD) of control *Rattus norvegicus* gavaged with distilled water versus an experimental group gavaged with 30mg of *Hoodia gordonii*/mL of distilled water *(P* = 0.198).

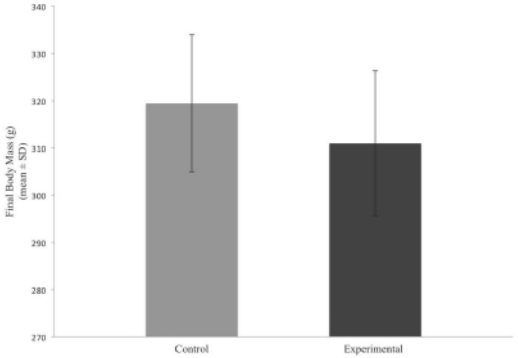

Figure 5. Comparison of average final body mass (± SD) of mean daily food consumption by control *Rattus norvegicus* gavaged with distilled water versus an experimental group gavaged with 30mg of *Hoodia gordonii*/mL of distilled water *(P* = 0.364).