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Effects of Citrus Aurantium Herb Extract on Dipsogenesis is Dose-Dependent in Rats

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Abstract

Currently, many herbal compounds have been evaluated for their use in weight loss diets. However, it is necessary to establish a link between the substance and the regulation of nutrient metabolism and thirst mechanism for the treatment of overweight. Our results showed that there was a significant decrease in food intake and transient loss in body weight in animals treated with *Citrus aurantium* herb extract at doses of 31.25 mg, 62.5 mg and 125 mg per 100 mg body weight per day. Fluid intake increased with dose indicating that *Citrus aurantium* effects on dipsogenesis are dose-dependent. Therefore, *Citrus auratium* can affect the thirst mechanism in rats. *Citrus aurantium* herb extract at a dose of 125 mg per 100 g body weight per day can have toxic effects in rats. Further studies are needed on treatment of obesity with *Citrus aurantium*.

Keywords: *Citrus aurantium*, Thirst mechanism, Rat

INTRODUCTION

Many nutraceuticals and herbal compounds have been evaluated for their use in weight loss diets in humans. These include herb extracts of *Citrus aurantium* ("bitter orange") standardized to 4 to 6% synephrine **[5]** and many other constituents that have various adrenergic effects. Possibly many people have used bitter orange extract-containing products without experiencing adverse events **[9]**. Synephrine is currently marketed, usually in combinations with other drugs such as caffeine, as an over-the-counter stimulant and weight-loss-promoting dietary supplement for oral consumption **[4]**. There is now ample evidence that synephrine exerts most of its biological effects as an agonist (i.e., stimulating) of adrenergic receptors, with a distinct preference for the α1 over the α2 sub-type. However, the effect of synephrine on these receptors is relatively low (i.e., relatively high concentrations of the drug are required to activate them). Synephrine has a weaker effect on adrenergic receptors of the β-class (regardless of sub-type) than on α-receptors **[8]**. Water is a vital nutrient for the body because it helps to keep the body temperature down, reducing sweating and dehydration, and serves as a transport mechanism in the body for carrying nutrients. Thus, there is a need to establish a link between the regulation of nutrient metabolism [**2**] and water intake in overweight treatment. In contrast to the numerous studies of foods, investigations of the control of fluid consumption in drinking bouts have been relatively sparse. Thus, in view of the complexity of the study of dipsogenesis, the objective of this work was to determine the possible alterations in dipsogenic responses associated with *Citrus aurantium* herb extract (dose-response curve) and its effects on food intake and mean weight change in rats.

MATERIAL AND METHODS

The study was approved by the institutional ethical committee (CEUA 2649). The experiments were carried out in groups of male Wistar Hannover rats with eight weeks of age (CEMIB-UNICAMP). The rats were housed under controlled climatic conditions, in accordance with current international bioethics and biosafety norms for animal experimentation and with the guidelines of the Brazilian College of Animal Experimentation (COBEA). For 5 days, at 9:00 a.m., each rat received by gavage 3 ml vehicle (filtered water) in the control group (n=10) *vs.* 31.25, 62.5 or 125 mg *Citrus aurantium* herb extract (Pharma Nostra) per 100 g body weight per day **[1]** (dose-response curve) diluted in 3 ml vehicle in the test groups (n=10). The animals were allowed free access to tap water and standard rat chow (Nuvilab Radiated - Nuvital Nutrientes S/A, Brazil) in the assessment period.

RESULTS

In the *Citrus aurantium*-treated groups, the effects (concentration independent) observed were increase in locomotor activity, piloerection, tachycardia and exophthalmia. **Figure 1** shows water and food intake and body weight change per 100 g body weight rats and curve dose-response. Mean ±SD (ANOVA and Bonferroni *t*-test *p≤ 0.05):

I. Pellet consumed in g: **[1a] - Control** (5.33 ± 0.43 at 24 h); (5.55 ± 0.52 at 48 h), (5.28 ± 0.43 at 72 h), (5.84 ± 0.86 at 96 h). **[1b] – 31.25 mg** (4.57 ± 0.86* at 24 h); (4.70 ± 0.32* at 48 h), (4.57 ± 0.26* at 72 h), (4.88 ± 0.25* at 96 h). **[1c] – 62.5 mg** (3.35 ± 0.39* at 24 h); (4.60± 0.44* at 48 h), (4.70 ± 0.43* at 72 h), (4.97* ± 0.26 at 96 h). **[1d] – 125 mg** (4.72 ± 0.39* at 24 h); (4.62 ± 0.49* at 48 h), (4.98 ± 0.46 at 72 h), (5.16 ± 0.45* at 96 h).

II. Water intake in ml: **[2a] - Control** (6.92 ± 0.77 at 24 h); (6.74 ± 0.87 at 48 h), (6.72 ± 1.45 at 72 h), (7.17 ± 1.55 at 96 h). **[2b] – 31.25 mg** (8.31 ± 0.44* at 24 h); (8.28 ± 0.40* at 48 h), (8.09 ± 0.48* at 72 h), (7.24 ± 0.80 at 96 h). **[2c] – 62.5 mg** (10.17 ± 1.12* at 24 h); (8.47 ± 0.33* at 48 h), (8.96 ± 0.79* at 72 h), (8.36 ± 0.65* at 96 h). **[2d] – 125 mg** (10.95 ± 1.64* at 24 h); (10.90 ± 1.96* at 48 h), (11.53 ± 1.84* at 72 h), (11.15 ± 2.15* at 96 h).

III. Body weight per 100 g: **[3a] - Control** (0.64 ± 0.66 at 24 h); (0.32 ± 0.29 at 48 h), (0.23 ± 0.18 at 72 h), (0.46 ± 0.38 at 96 h). **[3b] – 31.25 mg** (- 0.83± 0.95* at 24 h); (2.17 ± 1.09* at 48 h), (0.75 ± 0.36* at 72 h), (0.37 ± 0.17 at 96 h). **[3c] – 62.5 mg** (-1.91± 0.99* at 24 h); (1.87 ± 1.13* at 48 h), (0.60 ± 0.35* at 72 h), (0.57 ± 0.40 at 96 h). **[3d] – 125 mg** (- 0.52± 0.40* at 24 h); (-0.34 ± 0.69* at 48 h), (0.38 ± 0.48 at 72 h), (0.63 ± 0.70 at 96 h).

DISCUSSION

The data showed that there was a significant decrease in food intake in animals treated with *Citrus aurantium* herb extract, along with a transient weight loss proportional to the dose (mg) of *Citrus aurantium* herb extract given, in line with that reported in the literature **[7]**. The biological effect exerted by the administration of *Citrus aurantium* herb extract can involve leptin, which reduces food intake by reducing other orexin peptides or increasing thermogenesis and quenching appetite **[6]**. What should be considered, the effective concentration when combined with thirst mechanism results in rats. Because the increase in water intake did not accompany eating behavior at different doses in treated groups in mg herb extract *Citrus aurantium* per 100-g body weight per day, even above the levels of control animals. *Citrus aurantium* herb extract is an adrenergic stimulant, which may have three modes of action: direct interaction with specific receptors, and/or indirect action by stimulating release of neurotransmitters, and/or a mixed action involving both **[3]**. Still, for thirst, the excitatory signals

that initiate a drinking bout are established: increased systemic plasma osmolality, decreased plasma volume, and increased blood levels of angiotensin II (Ang II), or some combination of the three **[3]**. Initially, *Citrus aurantium* can stimulate the central nervous system including hypothalamus and cell bodies in the area to express high concentrations of Ang II receptor type 1 (AT1), which respond rapidly to an AngII stimulus and enhance the dipsogenic effect **[12]** depending on the dose of *Citrus aurantium* herb extract. The paraventricular and the supraoptic nucleus are considered osmoregulatory centers and the vasopressin sends signals (V1 receptors) to the hypothalamus directly or indirectly to stimulate the production of corticotropin-releasing hormone for the hypothalamic neurons **[10]**. In any case, *Citrus aurantium* herb extract can activate the hypothalamic pituitary-adrenal axis through primarily central mechanisms, i.e., those that involve the paraventricular nucleus and/or its afferents, as well as other brain areas potentially involved in a variety of neuronal responses and behaviors **[11]**. The increase or decrease in water intake can suggest that the interaction of *Citrus aurantium* herb extract with the osmoreceptors is dose-dependent.

CONCLUSION

Further investigations on the association of cell signaling pathway related to leptin and Ang II hypothalamic receptors and pharmacophysiology studies are needed before *Citrus aurantium* herb extract is used in overweight treatment.

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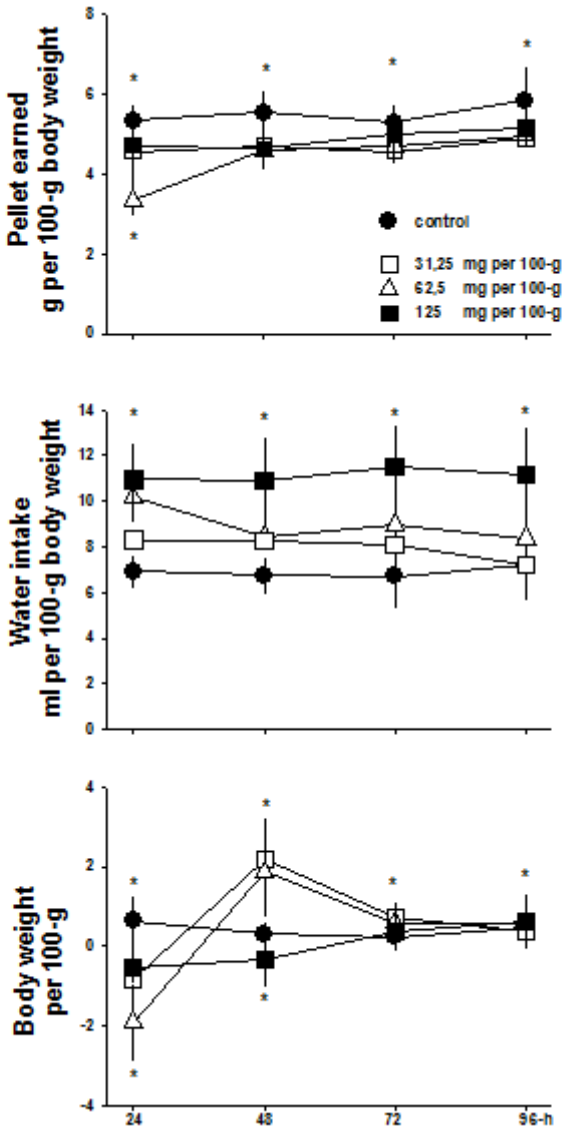

Figure 1 TIME-RESPONSE CURVES FOR DIFFERENT DOSES OF CITRUS AURANTIUM IN REGARD TO WATER AND FOOD INTAKE AND BODY WEIGHT CHANGE IN RATS Comparisons were made between vehicle alone in control group (n=10) *vs.* 31.25 mg, 62.5 mg or 125 mg *Citrus aurantium* herb extract in test groups (n=10), for results recorded at 24, 48, 72 and 96 h and expressed in milliliters and/or grams per 100 g body weight. Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) for repeated measures and Bonferroni's *t*-test to determine differences between the groups of animals. A p value ≤ 0.05 (*) was considered significant.

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