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Original Article

A STUDY COMPARING THE ANORECTIC ACTIVITY OF HOT AND COLD AQUEOUS EXTRACTS OF *DOLICHOS BIFLORUS LINN*. SEEDS IN FREELY FEEDING RATS

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ABSTRACT

Objective: The aim of the study was to evaluate the anorectic activity of hot (pre-cooked under pressure) and cold (soaked overnight) aqueous extracts of the seeds of *D. biflorus* in freely feeding Wistar albino rat model.

Methods: Hot extract at a dose of 4 % w/v was given to the group 1 and cold extract at a dose of 4 % w/v was given to group 2 while group 3 was kept as the control group. Food intake and analysis of behavioral satiety sequence (BSS) was assessed after 4 w of extract administration whereas body weight was recorded both before and after treatment.

Results: Cold extract group showed significant anorectic activity ($p<0.05$) but not the hot extract group. The cold extract group, unlike the hot extract group also exhibited an acceleration of the BSS indicating that the anorectic activity is primary.

Conclusion: The study has thus brought out the potential appetite suppressant activity of the cold extract of *D. biflorus* seeds.

Keywords: *Dolichos biflorus*, Anorectic, Behavioral satiety sequence, Food intake

INTRODUCTION

Obesity is a global health burden and a survey done in the Unites States population revealed that in 2009-2010, the prevalence of obesity was 35.5% among adult men and 35.8% among adult women [1]. The prevalence in children and adolescents was also found to be as high as 16.9% [2]. It is well known that excess body fat is associated with the development of various chronic conditions such as heart disease, metabolic syndrome, type 2 diabetes and dyslipidemia [3]. Prevention of obesity is, therefore, a major health priority globally. Lifestyle modification is one of the important measures to reduce body weight. This includes changes in diet like an increase in dietary fibre, reduction in both energy density and glycemic load which are thought to be particularly important in addition to increased physical activity [4]. Currently, there are only few drugs available for the treatment of obesity. Anti-obesity drug development history is far from glorious. Many anorectic drugs have been withdrawn from the market due to serious adverse effects. It is increasingly recognized that herbal compositions are useful in the prevention of obesity.

Dolichos biflorus, a legume, which is commonly known as "Horse gram", is native to most parts of India and belongs to the family *Fabaceae*. Seeds are the edible part and are used both as food and fodder. The methanolic extract of *D. biflorus* has hypolipidemic activity in high-fat diet fed rats [5]. It has also been proved that *D. biflorus* seeds have anti-adipogenic activity by inhibiting adipogenesis and intracellular lipid accumulation in *in vitro* assay [6]. But there are no studies done to evaluate the effects of *D. biflorus* on food intake and BSS in normal freely feeding rat model. Moreover, being an edible product, it is cooked before consumption and cooking may alter the phytochemical constituents and modify its actions. However, studies evaluating the effects of cooking on the actions of *D. biflorus* seeds have not been reported in the literature. Therefore, our study was designed to compare the appetite suppressant actions of hot (pressure cooked) extract with cold (overnight soaked) extract of *D. biflorus* seeds in freely feeding rat model.

MATERIALS AND METHODS

Healthy adult male albino rats of Wistar strain with a weight approximately 160-200 g were used in the study. They were housed in standard polypropylene cages under room temperature 25±2^{0}C and relative humidity of 45-55 %. They were exposed to 12:12 h light-dark cycle. The rats were fed with standard rat pellet diet and water *ad libitum*. The institutional animal ethics committee approved the protocol of the present study (approval no: 174/2012) and all the procedures were done in accordance with the ethical guideline on animal experimentation.

The D. biflorus seeds were obtained from the local market in Coimbatore and identified by a qualified botanist. A voucher specimen (Pha/02/2013) is stored in the department of Pharmacology, PSG institute of medical sciences and research, Coimbatore, TamilNadu, India. "Hot extract" was obtained by boiling the seeds under pressure for 15 min, which was then cooled before administration. The water in which the seeds were soaked overnight was used as the "Cold extract". Animals were divided into 3 groups, each consisting of 6 rats. The animals were randomly allocated to either group.

Treatment Group 1: given hot extract at a dose of 4% w/v in drinking water [7]

Treatment Group 2: given cold extract 4% w/v in drinking water

Control Group 3: drinking water

Control and treatment group rats were housed separately as 3 rats per cage using 2 cages for each group. Animals in all groups were weighed prior to extract administration at the start of the study. Drinking water was replaced with hot and cold extracts of *D. biflorus* seeds in the respective treatment groups for a period of 4 w. Freshly prepared extracts were given daily. At the end of 4 w, all the animals in each group were weighed individually.

Measurement of 24-hour food intake

After 4 w of treatment, the food intake assessment was also done. At 8 AM, after the animals were weighed, the pre-weighed amount of food was left in cages to assess the food intake over 24 hour period. The weight of left-over food was measured the next day at 8 AM. In order to obtain accurate measurements, the spilled over food at the bottom of the cage was returned to the left-over food before being

weighed. The amount of food intake was determined by the difference in weight of pre-weighed food to left-over food.

Measurement of behavioral satiety sequence

Natural satiation is associated with a predictable transition from feeding through grooming to resting. This orderly sequence is called behavioral satiety sequence (BSS). This sequence reflects the operations of natural physiological processes underlying satiety. BSS is produced by the presence of a caloric load in the gut that in turn triggers pre-absorptive satiety factors [8].

Evaluation of BSS was done after the measurement of food intake at the end of the treatment period. The animals were fasted overnight and the next morning, each animal was observed separately (one observer per animal) after placing food and water in the individualized observing cage for a duration of 60 min, without any external noise or movement during which they were left undisturbed. Animal behavior was recorded every one minute for 60 min. Behavior of the animal was classified into feeding (animal at hopper trying to obtain food, chewing, gnawing or holding food in paws), drinking (animal licking spout of water bottle), grooming (animal scratching, licking or biting any part of its anatomy), resting (animal curled up, resting head with eyes closed) and other (any other behavior or activity including locomotion, sniffing, reading, immobility when aware or signs of sickness behavior). Data observed were collated into 10-minute period bins for display. The time spent on feeding and the time bin when the transition from eating to resting occurred was calculated and compared between control and treatment groups.

Statistical analysis

All the data were expressed as a mean+standard deviation. The data was entered in Excel and analyzed using SPSS software version19. The difference in body weight before and after treatment in each of the groups was analyzed using "paired t test". One way ANOVA with *post hoc* LSD test was done to detect if there was any statistically significant difference in the food intake between the three groups. P value<0.05 was considered significant.

In BSS, the behavior was noted every one minute for a total duration of 60 min. It was grouped into feeding, drinking, grooming, resting and other. % of each behavior in every 10 min was calculated for each animal in all the groups and % of total behavior calculated as a group mean for every 10 min. Data was thus collated into 10-minute period bins for display (Time bin 1 is 0-10 min, 2 is 10-20 min, 3 is 20-30 min, 4 is 30-40 min, 5 is 40-50 min and 6 is 50-60 min) and this was analyzed qualitatively.

RESULTS

It was found that there was an increase in the body weight in all the three groups over the duration of 4 w from the start of the study. Using paired t-test, the increase in body weight was statistically significant in both the hot extract and control groups while in the cold extract group, the change in body weight observed at the end of the study was not statistically significant.(p>0.05) (table 1).

Table 1: Comparison of body weight of the three groups expressed as mean±SD deviation

Groups	Body weight (g)		p-value
	Before mean±SD	After mean±SD	
1	191.6±65.7	241.8±28.8	0.02*
2	190.6±74.0	232.3±51.8	0.16
3	214.8±59.6	253.5±29.3	0.04*

n=6 in each group. The observations are mean±SD body weight measured at baseline and after 4 w of treatment. p value<0.05 is significant

The mean 24-hour food intake of the animals treated with the cold extract was observed to be the lowest (fig. 1, 2). Using One way ANOVA and LSD *post-hoc* analysis to compare the mean food intake between the three groups, the cold extract group showed a statistically significant decrease in food intake over 24 hour period (p=0.03) compared to the control group. But a similar decrease noted in the hot extract group, was not statistically significant (table 2).

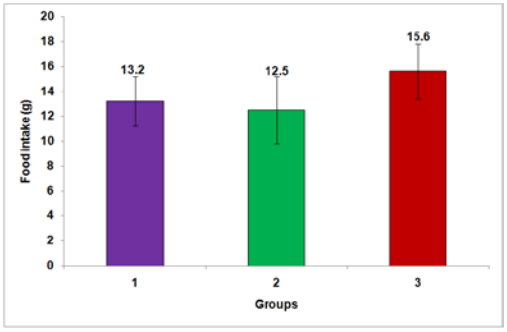

Fig. 1: Food intake of the three groups after treatment expressed as mean±SD

The columns represent mean food intake in grams (g) and the error bars indicate SD, n=6 per group

The control group displayed a normal BSS. The transition from feeding through grooming to resting occurred in the fourth time bin. The BSS in the hot extract group was found to be altered but not accelerated as the time spent on food intake in the earlier time bins (time bins 1 and 2) was higher than the control group while there was a reduction in the feeding time in the time bins 5 and 6. Also, the transition from feeding to resting was noted in the fourth bin which was same as that of the control group (fig. 3, 4).

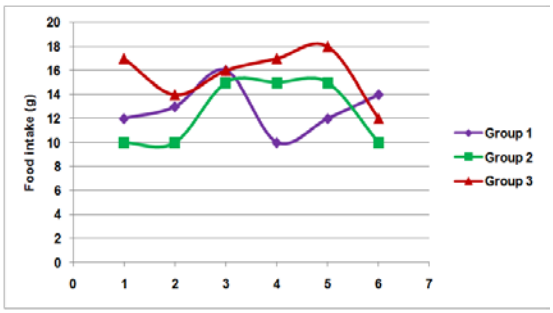

Fig. 2: Scatter plot chart comparing the food intake between the three groups

Table 2: *Post hoc* LSD test to compare the means of food intake between the three groups

Between-groups comparison	Mean difference	p-value
Group 1 & Group 2	0.33	0.80
Group 2 & Group 3	3.16	0.03*
Group 3 & Group 1	2.83	0.06

The observations represent between group comparisons of food intake done using one-way ANOVA and post-hoc LSD. p<0.05 is significant

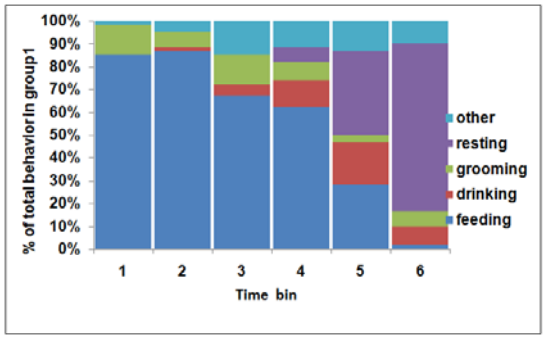

Fig. 3: BSS of group1

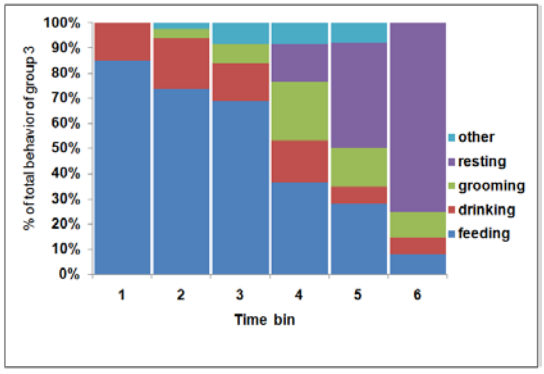

Fig. 4: BSS of group3

Contrarily, the satiety sequence in the cold extract group revealed that the time spent on food intake was consistently lower in all the time bins compared to the control, and also the point of transition happened in the third time bin, earlier than that of control. There was an apparent shift of the sequence to the left, demonstrating acceleration of the BSS in the group treated with cold extract (fig. 4, 5).

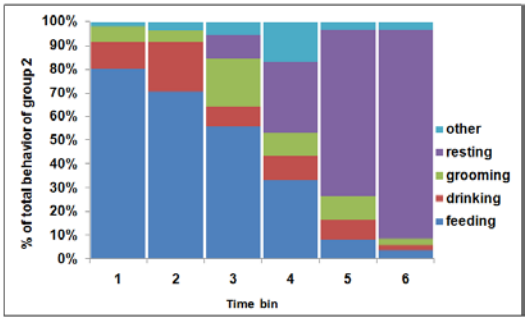

Fig. 5: BSS of group2

DISCUSSION

Obesity characterized by excess adipose tissue mass in the body can be the result of excess energy intake. It is well known that high caloric intake and reduced physical activity increases the adipose tissue mass through the process of adipogenesis and that anorectic drugs primarily act by reducing the high energy intake by suppression of appetite [9]. Various edible plant products have been shown to possess anorectic properties [10], which unlike the anorectic drugs fenfluramine, sibutramine are free of adverse effects.

In the present study, we have investigated the effect of orally administered aqueous extracts of *Dolichos biflorus* seeds on food intake and behavioral satiety sequence of freely feeding rats. All the animals in the three groups exhibited an increase in body weight over the period of four weeks. This is attributed to the fact that the animals chosen were young adults in the growing phase and were not on any food restriction. But the study has shown that the cold extract significantly attenuated the rise in body weight in the treated animals since there was no statistically significant difference between the mean body weight at baseline and that obtained after treatment, unlike the other two groups which demonstrated a statistically significant increase in body weight after 4 w. This could be explained by the significant hypophagic effect of the cold extract as the mean food intake measured in this group was significantly lower compared to the control group, indicating potential appetite suppressant or anorectic activity. However, the hot extract neither produced a significant reduction in food intake when compared to controls nor significantly reduced the rise in body weight of the treated animals (table 1, 2).

The mechanisms underlying the anorectic activity of *D. biflorus* seeds are not fully understood, though it is probable that this could be due to inhibition of ghrelin secretion [6]. Ghrelin, secreted by the X/A like endocrine cells of the gastric fundus, is a key hormone that regulates food intake [10]. In the CNS, ghrelin stimulates the hypothalamic production of neuropeptide Y (NPY) and Agouti protein (AgRP) by influencing the mitochondrial uncoupling proteins [12]. These neuropeptides serve as potent orexigenic stimulus mediating the hyperphagia, increased adiposity and weight gain responses of ghrelin [13]. Lower circulating ghrelin is associated with reduced appetite and reduced food intake [14].

Anorectic drugs can be either primary or secondary. A primary anorectic is a drug that acts by accelerating but maintaining the integrity of BSS e. g. fenfluramine. A secondary anorectic acts by disrupting the BSS by inducing nausea or by the interference of taste mediated positive feedback. Therefore, there is a need to differentiate treatments that suppress intake by primary action from secondary action [15]. Our study has shown that *D. biflorus* cold extract does not disrupt but accelerates the BSS indicating that the extract acts by the primary anorectic mechanism. (fig. 5) Thus our study highlights that the anorectic potential of *D. biflorus* seeds rests primarily with cold extract and not the hot extract as revealed by the body weight changes, mean food intake and BSS of the study animals. The reason for this observation could probably be due to alteration of the nutrient composition of legumes during cooking under pressure [16].

CONCLUSION

The study has brought out that the cold extract of *D. biflorus* seeds possesses anorectic activity and in addition, the BSS analysis has proved it to be a primary anorectic. This signifies that *Dolichos biflorus* seeds have potentially beneficial effects in the treatment of obesity.

CONFLICTS OF INTERESTS

There are no conflicts of interest. No funding sources involved

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