DYPTERYX ALATA VOGEL SEEDS MAY IMPROVE ANTHROPOMETRIC AND BEHAVIORAL PROFILE OF WISTAR RATS

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ABSTRACT

*Dipteryx alata* Vogel (DA), popularly known as *baru*, is a native tree from the Brazilian Cerrado and belongs to the family Fabaceae. It possesses a seed (almond), covered by a sweet yellow pulp and a brown shell that is rich in proteins, with an important amount of essential amino acids, unsaturated fatty acids, mainly oleic acid, fiber, vitamins and minerals. The objective of this research was to investigate the effects of baru almonds on the anthropometric profile and behavioral of Wistar rats. Animals weighting 180g-220g were divided randomly in G1 (n=10) that was fed water and rat food *ad libitum* and G2 (n=10) that was fed water and rat food supplemented with DA 40% *ad libitum* for 40 days. Our results showed that *Dipteryx alata* reduce food intake and may help prevent the increase in body weight and deposition of visceral fat as well as it may help in the reduction of the anxiety behavior. Baru seeds may play an important role in the agro-
industry due to its nutritional properties and flavor and may work as adjuvants in the treatment of several pathologies such as obesity.

**KEYWORDS:** *Dipteryx alata*, obesity, behavior.

**INTRODUCTION**

The study of biomes as the Brazilian Cerrado is important to assist in the preservation of the biodiversity of these ecosystems. It is considered as one of the richest area of savannah in the world due to its flora including exotic native fruit and seeds that have being recognized as possessing unique flavours and important nutritional value. In addition to this, plants that exhibit medicinal properties can promote benefic physiologic actions in the organism and play an important role in the health of individuals and communities. These plants often are source of bioactive compounds such as tannins, flavonoids, alkaloids, and phenolic components that are responsible for the health properties. For these reasons, several researchers have been working with plants and their bioactive compounds in order to clarify the medicinal applications. Since antiquity, most of drugs were acquired from nature in a direct or indirect way.[1-4]

*Dipteryx alata* Vogel (DA) is one of the native fruit from the Brazilian Cerrado, belongs to the family Fabaceae and is popularly known as *baru*. It possesses a seed (almond), covered by a sweet yellow pulp and a brown shell. This seed is rich in proteins, with an important amount of essential amino acids, unsaturated fatty acids, mainly oleic acid, fiber, vitamins and minerals.[5-7]

Due to the presence of several bioactive compounds, the intake of almonds and seeds may be important in the prevention of chronic degenerative diseases such as obesity, diabetes, metabolic syndrome and cardiovascular diseases that are among the main cause of death in the world.[8-10]

Obesity is increasing worldwide and reached the position of most prevalent nutritional disorder. The deposition of fat mainly in the abdominal region leads to an inflammatory process that result in the release of several cytokines that increase the risk of developing diabetes, cardiovascular diseases and cancer. Several therapeutic approaches have been thought but many show little efficiency. The use of healthy foods as seeds may help in this approach.[11-12]
The use of native plants for functional or medicinal purposes, besides helping the preservation of biome, may be an interesting alternative to obtain newer and tasteful products that may work as a complement in the nutritional needs. Based on this, the objective of this research was to investigate the effects of baru almonds on the anthropometric profile and behavioral of Wistar rats.

METHODS

Preparation of the rat feed supplemented with baru seeds
Baru seeds were toasted in air circulating oven at 130°C (for 30 minutes). After that, the supplemented rat food was prepared with a proportion of 40% of toasted seeds and 60% of commercial feed. Seeds and rat feed were crushed and mixed and molded into pellets that were dried in an air circulating oven at 65°C for about 8 hours, stored in polyethylene packaging, and refrigerated at 5°C until its utilization.

Ethics
This study was approved by the Animal Research Ethics Committee of the Faculty of Food Technology (FATEC), Marília - SP, Brazil (protocol number 001/2016). Animals were fed and watered ad libitum during the experimental period and were cared for according to the recommendations of the Canadian Council’s “Guide for the care and use of experimental animals”.

Animal groups
Wistar rats (males) weighing 180g-220g were housed in collective cages under a dark/light cycle of 12 hours, room temperature of 22 ± 2°C, and relative air humidity of 60 ± 5% at the Faculty of Food Technology (FATEC), Marília - SP, Brazil. After seven days of acclimation to laboratory conditions, the animals were divided randomly in G1 (n=10) that was fed water and rat food ad libitum and G2 (n=10) that was fed water and rat food supplemented with DA 40% ad libitum.

The weight of the animals was evaluated each three days and the consumption of the rat feed was based on the leftovers found every day.

Behavioral test
At the end of the experimental protocol (40th day), the male rats underwent the Elevated Plus Maze (Boerngen-Lacerda et al.[13] and Blanchard et al.[14]) for behavioral assessment with the
objective of evaluating the anxiety index in rats from the control group (G1) and the animals treated with DA almonds 40% (G2).

The structure of the Elevated Plus Maze (EPM) is wood made and fits 50 cm from the ground. It includes two open and opposed arms, measuring 50x10 cm, and two enclosed, with 50 x 10 x 40cm. There are also platforms with the same extent of the open arms that cross them perpendicularly, and delimits a central area of 10 cm². The animals were placed in the EPM for 5 minutes in order to evaluate the frequency and the time spent in the center, in open and in closed arms.

Anthropometric evaluation
In the 41\textsuperscript{0} day of the experimental protocol, after the euthanasia process, the visceral fat was removed from abdominal region it was weighed. The rats were euthanized with a lethal intraperitoneal injection of thiopental (200 mg/Kg).

Statistical analysis
For the Statistical analysis, ANOVA complemented with Tukey test was used to comparison among the groups and Correlation Analysis was applied to evaluate the correlation among groups in time and frequency in the Elevated Plus Maze. Variables were presented as mean and standard error mean, adopting a 5% level of significance.

RESULTS
Our results show that food consumption, body weight at the end of the experimental protocol and visceral fat decreased significantly in the groups treated with baru seeds. Body weight at the beginning is not significant when comparing the G1 and G2 (Table 1).

Table 1: Mean and standard deviation of the food consumption and anthropometric parameters in the Control Group (G1) and group treated with DA (G2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1</th>
<th>G4</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food consumption (mg/kg)</td>
<td>124.4±8.94</td>
<td>105.53±6.72</td>
<td>0.000*</td>
</tr>
<tr>
<td>Body weight 1</td>
<td>158.75±22.23</td>
<td>160.4±21.27</td>
<td>0.712</td>
</tr>
<tr>
<td>Body weight 2</td>
<td>358.3±21.66</td>
<td>330.4±17.45</td>
<td>0.0498*</td>
</tr>
<tr>
<td>Visceral fat (g)</td>
<td>15.91 ± 5.88</td>
<td>10.01 ± 2.26</td>
<td>0.0467*</td>
</tr>
</tbody>
</table>

BW1: Body weight at the beginning of the treatment (g); BW2: Body weight at the end of the treatment (g). *Level of significance: 5%.
Results for the behavioral test (Table 2) show that the use of the baru seeds did not significantly modify the parameters of time and frequency in the open and closed arm and in the center and both groups spent more time in the center.

**Table 2: Mean and standard deviation of the Behavioral testing of Control Group (G1) and group treated with DA (G2).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Position</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G1</td>
</tr>
<tr>
<td>Time</td>
<td>Open</td>
<td>13.55 ± 3.78 A&lt;sup&gt;1&lt;/sup&gt;b&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Center</td>
<td>5.41 ± 3.13 Aa</td>
</tr>
<tr>
<td></td>
<td>Closed</td>
<td>25.27 ± 16.46 Ab</td>
</tr>
<tr>
<td>Frequency</td>
<td>Open</td>
<td>6.75 ± 3.62 Aa</td>
</tr>
<tr>
<td></td>
<td>Center</td>
<td>12.75 ± 5.31 Ab</td>
</tr>
<tr>
<td></td>
<td>Closed</td>
<td>7.00 ± 2.98 Aa</td>
</tr>
</tbody>
</table>

<sup>1</sup>Means followed by at least one same capital letter do not differ according to the comparison among the groups; <sup>2</sup>Means followed by the same lower case letter do not differ according to the comparison in the group. *Level of significance: 5%.

The correlation analysis showed that the group G1 spent less time but exhibited higher frequency in the central position and G2 spent more time in the open and closed position but the frequency was lower.

**Table: 3. Correlation between mean time and frequency of the rats in the labyrinth according to the apposition and the groups.**

<table>
<thead>
<tr>
<th>Position</th>
<th>G1 r</th>
<th>p-value</th>
<th>G2 r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open</td>
<td>0.04</td>
<td>0.9327</td>
<td>-0.71</td>
<td>0.0500</td>
</tr>
<tr>
<td>Center</td>
<td>-0.52</td>
<td>0.0289</td>
<td>-0.76</td>
<td>0.0483</td>
</tr>
<tr>
<td>Closed</td>
<td>-0.49</td>
<td>0.2165</td>
<td>-0.67</td>
<td>0.0663</td>
</tr>
</tbody>
</table>

*p<0.01, according to Tukey Test.

**DISCUSSION**

Our results show that the use of DA seeds may collaborate with the reduction of abdominal fat and body weight in Wistar rats and also reduce the food intake as observed in the treated group (G2). Nowadays, the occurrence of obesity has reached ever smaller ages and its incidence has risen so alarmingly that it can be considered a public health problem. Many are the causes of the increase of this pathology like the increase of the intake of foods with high
sugar and fat contents, physical inactivity and behavioral patterns like the increase of anxiety.\textsuperscript{15-17}

The increase in the body weight and abdominal fat increases oxidative stress, inflammation processes, dyslipidemia, hypertension, insulin resistance/diabetes, cardiovascular diseases, and the complications of these conditions that are directly linked to the morbidity and mortality.\textsuperscript{11, 18-19} On the other hand, the atherogenic process is a phenomenon that occurs slowly and may start years before the clinical manifestation. For this reason, many researchers are motivated to study and develop new therapeutic approaches, such as the use of plants that may improve the risk factors before the atherosclerotic process becomes an irreversible condition. For example, the endothelial dysfunction that is strongly associated with oxidative stress represents the early stage of the disease and may be reversible as well as obesity and insulin resistance. The release of inflammatory cytokines and other markers such as Interleukin-1β (IL-1 β ), IL-6, Tumor Necrosis Factor-α, and C-reactive protein may also be reversible.\textsuperscript{11, 20-22}

The Elevated Plus Maze (EPM) test is widely used to investigate the psychological bases of anxiety. Usually an animal without any treatment tends to remain for a longer time in the closed arms that represents an exposition to naturally threatening situations, which in the above model are represented by height and open space. This may explain the greater tendency to avoid exploring the open arms. The presence of closed arms gives to the animal a safer environment against potentially dangerous stimuli such as the presence of predators. The presence of substances that reduce anxiety makes the animal feel calmer and consider the environment less threatening.\textsuperscript{23-24}

Usually the animal is observed in the EPM for 5 minutes and is allowed to explore the structure freely in this period of time. Exploring the open arms occurs only in 20 to 25\% of the time, indicating a behavior to avoid exploring these arms. In our study, the correlation analysis showed that the animals treated with baru seeds tended to spend a longer period in the open and closed space, and the frequency was lower, indicating a lower level of anxiety. Animals treated with baru also spend a longer time in the central area what is considered as a decreasing of anxiety behavior. Some studies show that animals treated with anxiolytic substances increase the total time spent in the open arms, indicating that the pattern of tension and anxiety behavior was reduced and resulted in a longer stay in a place that, under normal
conditions, would indicate a stress situation due to height and lack of protection due to the absence of walls.\textsuperscript{[25-29]}

Several authors have shown the anxiolytic effects of plants in human and in animal models.\textsuperscript{[26,29-31]} Nevertheless we did not find studies in literature that showed the effects of DA seeds in the behavior of Wistar rats.

Although edible seeds and nuts contain high energy value, they are good sources of high quality lipids. The baru seed has a lipid content of approximately 40\% as well as other nuts commonly consumed by the population. Nevertheless, studies show that the intake of these products does not result in weight gain and obesity, and can often help weight loss. Furthermore, these foods are good sources of fiber and baru contains about 12\% of insoluble fibers that increase fecal bulk and decrease intestinal transit time, which makes it important to reduce the absorption of fats and sugars from the diet. Thus, seeds such as baru can be incorporated into the diet since they have high protein, lipid and fiber content.\textsuperscript{[32-34]}

Literature shows that some individuals may develop dysfunctional patterns of beliefs and perceptions of the world and themselves that start usually in childhood or adolescence due to psychologically harmful experiences in the family, friends or other close/significant people. These called maladaptive schemas may lead individuals to develop different kinds of psychological disorders and dysfunctional behavior that can trigger dysfunctional eating behaviors. Authors have shown high prevalence of dysfunctional patterns such as inhibition, abandonment, instability, and others that may negatively influence aspects of identity amongst individuals with obesity. Obese or overweight adolescents, when compared to normal weight adolescents, present insufficient self-control, emotional deprivation, alienation, social isolation, shame, and dependence. These characteristics may lead to the loss of control over eating. Therapeutic approaches that can help reducing anxiety and food intake may be very important in our modern societies where obesity is considered a public health problem.\textsuperscript{[35-39]}

**CONCLUSION**

Native fruit from Brazil may play an important role in the agro-industry due to the nutritional properties and flavor. Besides, these plants may work as adjuvants in the treatment of several pathologies such as obesity. Our results showed that \textit{Dipteryx alata} reduced food intake and
may help in the prevention of increase in body weight and deposition of visceral fat as well as it may help in the reduction of the anxiety behavior.

ACKNOWLEDGMENTS

We are grateful to Milton Groppo for having identified the plant used in this research.

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