

# Sodium benzoate, a food preservative, induces anxiety and motor impairment in rats

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## ABSTRACT

**الأهداف:** دراسة الخصائص السلوكية والتي تشمل القلق، والإعاقة الحركية لدى الفئران المعالجة ببنزوات الصوديوم.

**الطريقة:** أجريت دراسة خلال الفترة من يوليو حتى سبتمبر 2012م في مركز مختبر الحيوانات المركزي، جامعة Shiraz للعلوم الصحية، Shiraz، إيران. تم تقسيم الفئران إلى مجموعتين تلقت الماء المقطر وبنزوات الصوديوم 200 ملغ/كلغ/اليوم). تلقت جميع الحيوانات الغذاء اليومي لمدة 4 أسابيع. في نهاية الأسبوع الرابع، تم قياس كلا من المستوى الحركي ومستوى القلق في المتاهة المرتفعة واختبار الدوران.

**النتائج:** طبقاً للنتائج، استغرقت الفئران المعالجة ببنزوات الصوديوم وقتاً أقل في الذراع المفتوحة وأقل مداخل للذراع المفتوحة بالمقارنة مع مجموعة الشاهد  $p < 0.04$ . كما انخفض أداء الفئران المعالجة ببنزوات الصوديوم في القضيبي الدوران ووقت الركوب بشكل أقل من مجموعة الشاهد ( $p < 0.01$ ).

**خاتمة:** انخفض أداء الفئران المعالجة ببنزوات الصوديوم في المتاهة المرتفعة كمقياس للقلق. إضافة إلى ذلك، انخفض كلا من وقت الركوب في الدوران والسرعة الثابتة إشارة إلى الإعاقة الحركية.

**Objective:** To investigate the behavioral characteristics, including anxiety and motor impairment, in sodium benzoate (NaB) treated rats.

**Methods.** The study was carried out between July and September 2012 in the Laboratory Animal Center of Shiraz University of Medical Sciences, Shiraz, Iran. The rats were divided into 2 groups receiving distilled water and NaB (200mg/kg/day). All the animals received daily gavages for 4 weeks. At the end of the fourth week, anxiety, and motor function were assessed in elevated plus maze and rotarod test.

**Results.** According to the results, NaB-treated rats spent less time in the open arm and had fewer entrances to the open arms in comparison with the control group ( $p < 0.04$ ). Also, the performance of the NaB-treated rats in fixed and accelerating speed

rotarods was impaired, and the riding time (endurance) was lower than the control group ( $p < 0.01$ ).

**Conclusion:** The performance of the NaB-treated rats was impaired in the elevated plus maze, an indicator of anxiety. Their riding time in fixed and accelerating speed rotarods was decreased, indicating motor impairment.

*Neurosciences 2014; Vol. 19 (1): 24-28*

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Received 29th June 2013. Accepted 17th December 2013.

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With the increase in the production of processed, convenience, and fast foods, food additives have become increasingly important in modern food technology.<sup>1</sup> Sodium benzoate (NaB) ( $C_6H_5COONa$ ) is widely used as a food preservative and antimicrobial substance in a variety of products, such as salad dressings, pickles, vinegar, carbonated drinks (carbonic acid), jams and fruit juices (citric acid), and sauces. It is also used in other materials, such as certain medications and shampoos.<sup>2</sup> The NaB, a metabolite of cinnamon, is utilized for urea cycle disorders, suppresses inflammation, and switches the differentiation of T cells.<sup>3</sup> It is also permitted to be used in oral medicines and cosmetics.<sup>4,5</sup> There is evidence of the neuro-

**Disclosure.** This project was financially supported by the Research Vice-Chancellor of Shiraz University of Medical Sciences, Shiraz, Iran (Grant No. 91-6416).

pharmacological properties of NaB. In one case report,<sup>6</sup> NaB was used to relieve panic symptoms. Another report indicated that NaB upregulates the neuroprotective protein in Parkinson disease in astrocytes and neurons.<sup>7</sup> It has also been shown that NaB acts as an inhibitor of D-amino acid oxidase, and can block D-dopa-induced circling in rats. It has no effect on circling induced by L-dopa.<sup>8</sup> Activation of microglia and astrocytes generate a number of proinflammatory molecules that take part in the pathophysiology of many neurodegenerative disorders. A study by Brahmachari et al<sup>9</sup> explored the anti-inflammatory property of NaB in microglia and astrocytes. Experimental allergic encephalomyelitis (EAE) is the animal model for multiple sclerosis. The results of the study by Brahmachari and Pahan<sup>10</sup> suggest that NaB modifies encephalitogenic T cells at multiple steps, and that NaB may have a therapeutic significance in multiple sclerosis. Other studies have suggested that a high intake of NaB may contribute to symptoms such as Attention Deficit-Hyperactivity Disorder (ADHD) in college students, but further investigations are required.<sup>11</sup> Furthermore, NaB consumption has been directly linked with childhood hyperactivity.<sup>1,12-15</sup> Therefore, the present study was conducted to evaluate the behavioral activities, such as motor impairment and anxiety, of rats after NaB treatment. In order to evaluate anxiety and motor impairment after exposure to NaB, the rats' Elevated Plus-Maze (EPM) and performance in rotarod was monitored. The EPM is among the most popular behavioral models of anxiety, and is used to define brain regions and the mechanisms underlying anxiety-related behaviors.<sup>16</sup> The rotarod test is widely used to evaluate the motor coordination of rodents, and is especially sensitive in detecting cerebellar and striatal dysfunction.<sup>17</sup>

**Methods.** The study was carried out between July and September 2012 in the Laboratory Animal Center of Shiraz University of Medical Sciences, Shiraz, Iran. In the present study, 20 Sprague-Dawley adult male rats (180-230 g) were sampled from the Laboratory Animal Center of Shiraz University of Medical Sciences. The Ethics Committee of the University approved the use of the animal experiment under approval No. 91-6416, and all efforts were made to minimize animal suffering. Using simple random sampling, the animals were divided into 2 groups each including 10 rats. The first group received distilled water (solvent of the NaB) and the second group received NaB (Negar Azar, Qom,

Iran) (200mg/kg/day).<sup>18,19</sup> The animals were housed in plastic cages under a 12 hours/12 hours light/dark cycle at a room temperature of 22±2°C.

**Behavior was assessed in the EPM.** The EPM consisted of 2 open arms (50x10 cm<sup>2</sup>) without walls, and 2 closed arms (50x10 cm<sup>2</sup>) with 40 cm walls that extended from a common central platform (5x5 cm<sup>2</sup>). Small plastic boundaries (0.6 cm) were located along each side, and at the end of the open arms to minimize the possibility of falling down during the experiment. At the beginning of the EPM session, each rat was placed on the middle platform facing a closed arm. The behavior on the maze was recorded for 5 minutes using a camera. The behavioral measures were later scored from the recorded video by an experimenter who was unaware of the experimental state of each animal. The maze was cleaned after each animal experiment. The scored quantities included Open Arm Time (OAT), Closed Arm Time (CAT), Open Arm Entries (OAE), Closed Arm Entries (CAE), and General Motor Activity (GMA) or the total number of entries into both enclosed and open arms. When all 4 limbs of a rat were placed in the arm, the rat was considered to have entered an arm. When at least 2 front limbs were placed outside the arm, the animal was considered to have exited an arm. Percentage of time spent on the open arms and percentage of open arm entries have been reported as reliable measures of anxiety.<sup>16,20-22</sup> Also, the time spent in the center has been used to assess non-anxiety behavior, such as risk-assessment, but this evaluation is less common.<sup>23</sup>

A rotarod machine with automatic timers and falling sensors was used in this study. First, the rat was placed on a rod and the rod's surface was covered with hard chloroethylene, which does not permit gripping on the surface. The animals were pre-trained on the rotarod apparatus. The rats were tested first on the fixed speed rotarod protocol and on the accelerating rotarod protocol 2 days later. On the testing day, according to the fixed speed protocol, the animals were tested at 8 different speeds, including 12, 16, 19, 21, 24, 26, 28, and 38 rpm, for at most 60 seconds at each speed. The rats underwent the test 3 times at each speed with an interval of 20 minute between each trial. The latency to fall or endurance of each rat was also recorded. According to the accelerating rotarod protocol, the animals were placed on the rod whose rotation speed steadily increased from 4 to 40 rpm during a 300 second period. The endurance time or latency to fall was also recorded.<sup>24</sup>

**Statistical study.** The data was analyzed using analysis of variance (ANOVA) followed by Tukey's test.  $P \leq 0.05$  was considered statistically significant.

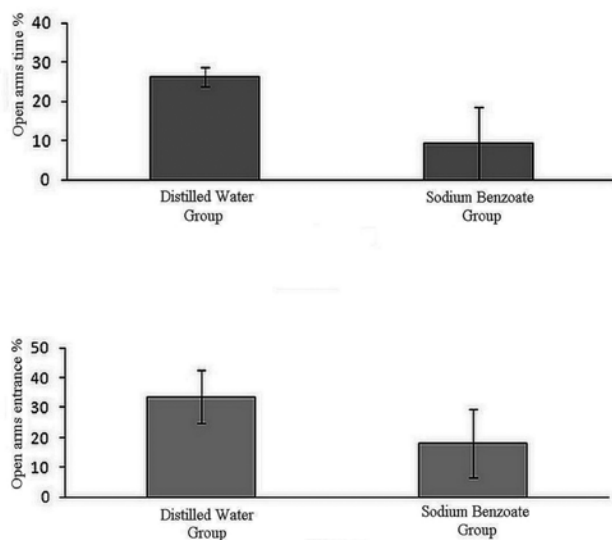
**Results. Elevated plus maze.** The results related to OAT and OAE, as measures of anxiety, revealed a significantly lower percent of OAT and OAE by NaB rats in comparison with the distilled water group ( $p < 0.04$ ). The results showed that after treating the rats with NaB, they spent less time (9.5% of the total time) in the open arm in comparison with the distilled water rats (26% of the total time) (Figure 1). The rats treated with NaB also showed fewer entrances to the open arms (18% of the total entrances) in comparison with the distilled water group (34% of the total entrances) (Figure 1).

**Rotarod test.** The performance of the NaB-treated rats was impaired in the fixed speed rotarod. In addition, their riding time (seconds) or endurance was 46 seconds at the lowest, and 26 seconds at the highest speeds. These measures were 59 seconds at the lowest, and 51 seconds at the highest speed in the distilled water group. The performance of the NaB-treated rats in the accelerating speed rotarod was also impaired, and their riding time (seconds) was 43% lower than that of the distilled water group (Figure 2).

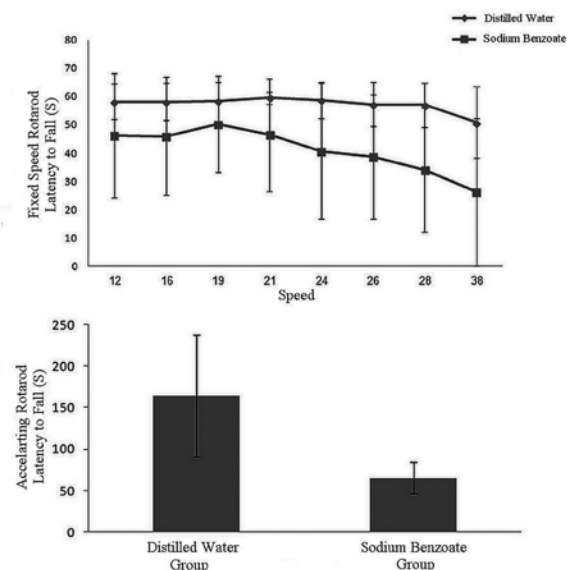
**Discussion.** The present study evaluated the behavioral characteristics of the rats after treatment with

NaB, and showed motor impairment and anxiety in the NaB-treated rats. There are many reports regarding food additives, such as NaB, which are associated with various side effects.<sup>25</sup> Different research has shown a significant relationship between the consumption of NaB by children and the onset of hyperactive behavior.<sup>26,27</sup> More than 35 years ago, Ben Feingold made his initial claims on the adverse effect of food additives on childhood behavior.<sup>27,28</sup> The main accepted effect of food additives, including NaB, on children is hyperactivity, impulsiveness, and careless behavior. The children who execute this behavioral pattern to a large degree are probably diagnosed with ADHD.

The motor impairment of the NaB-treated rats might also be related to inattentive behavior. However, different mechanisms can be considered by which NaB can influence motor impairment and anxiety. Metabolization of benzoate in the liver is performed by conjugation with glycine. Yet, using glycine in detoxification of benzoate results in reduction of the glycine level in the body, which can affect the metabolic process in which glycine is involved.<sup>29,30</sup> It has also been reported that a decrease of glycine leads to anxiety.<sup>30</sup> Moreover, low glycine levels in the body can cause depletion in glutamine. Astrocytic-derived glutamine is the precursor of the 2 most important neurotransmitters: glutamate, an excitatory neurotransmitter, and GABA, an inhibitory neurotransmitter. In addition to their



**Figure 1** - Mean  $\pm$  SD of the percentage of time spent in the open arms and percentage of the number of open arm entrances in the elevated plus-maze test in the control (distilled water), and sodium benzoate groups ( $p < 0.04$ ).



**Figure 2** - Rotarod performance. Mean  $\pm$  SD of the latencies to fall (or endurance) in the control (distilled water) and sodium benzoate groups tested on the fixed and accelerating speed protocols ( $p < 0.01$ ).

roles in neurotransmission, these neurotransmitters act as alternative metabolic substrates that enable metabolic coupling between astrocytes and neurons.<sup>31</sup> These 2 neurotransmitters (glutamate and GABA) play key role functions in the neuronal cycles of the brain, which are involved in motor activities and therefore the rotarod performance.

Another mechanism that might be responsible for the impairment in rotarod and elevated plus maze is zinc deficiency. The NaB can induce changes in the brain that significantly reduce the zinc level in the brain. This will eventually lead to behavioral changes in the mice.<sup>32</sup> Zinc deficiency has also been reported to be associated with cognitive and motor function impairment, depression, anxiety, and ADHD symptoms.<sup>33-35</sup> The NaB also effects gait and visual discrimination.<sup>36</sup> There is evidence that exposure to NaB results in developmental problems in axons of motor neurons and neuromuscular junctions in zebra fish larvae.<sup>37</sup> The NaB also induces inhibitory effects on the expression of inducible NO synthesis in astrocytes and microglia.

According to the Food and Drug Administration, the concentration of NaB as a preservative is limited to 0.1% of body weight. The International Program on Chemical Safety reported no unfavorable effects in humans at doses of 647-825 mg/kg of body weight per day.<sup>28</sup> Sodium benzoate is used in management of patients with urea cycle pathology (namely, hyperammonemia by reason of inherited errors of urea production). This treatment facilitates another way for nitrogen excretion, and 250-500 mg/kg body weight per day has been considered as a therapeutic dose prescribed over several years.<sup>38</sup> The clinical signs of toxicity are uncommon with this dosage, and restricted to anorexia and vomiting in most cases. The dose of NaB used in the present study was 200 mg/kg/day according to the study by Oluwole et al.<sup>18</sup> According to the results; this dose could induce some impairment in the rats.

One of the limitations of this study was the absence of data on NaB in the blood serum of the animals. It should be recommended to measure the concentration of NaB blood serum in future studies. The results of this research can be used for future study, focused on using the preservatives in human regimes.

In conclusion, treatment of the rats with 200mg/kg/day NaB impaired their performance in the EPM, indicating anxiety. The riding time in both the fixed and accelerating speed rotarod test was also decreased, indicating motor impairment.

**Acknowledgment.** *This research is a part of the thesis written by Mahboobeh Erfanizadeh, MSc student of anatomy,*

*Shiraz University of Medical Sciences (Grant No. 91-6416). The research was carried out at Histomorphometry and Stereology Research Centre. The authors would like to thank the Research Improvement Center of Shiraz University of Medical Sciences, and Ms. A. Keivanshekouh for improving the use of English in the manuscript.*

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