Neurobehavioral alterations in male rats exposed to Sodium Benzoate

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Abstract: The recent research studied the effect of different doses of exposure to sodium benzoate on levels of anxiety and fear, depression and anti- social behavior in male rats. Oral administration of 0%, 0.5 %(low dose) and, 2% (high dose).sodium benzoate to 45 male Wistar rats randomly, allotted into three groups of 15,were performed daily for 12 weeks treatment period. The animals were observed for neurobehavioral disturbance. Anxiogenic effect of sodium benzoate was evidently observed during EPM (elevated plus maze) and dark light transition tests. Moreover, noticeable effect of sodium benzoate on depression is expressed by prolonged immobilization during forced swim test. Impairment of social interaction test was also detected in treated rats. Our results strongly provides sufficient scientific evidence that a causal link truly exists between sodium benzoate and inflection of anxiety, depression-like behaviors and anti- social behavior in rats and points to the hazardous impact of sodium benzoate on public health.

1. Introduction:

A major market for sodium benzoate is as a preservative in the soft drink industry, as a result of the demand for high-fructose corn syrup in carbonated beverages. Sodium benzoate is also widely used as a preservative in pickles, sauces, and fruit juices (Srour, 1998). Sodium benzoate is also used in pharmaceuticals for preservation purposes (up to 1.0% in liquid medicines) and for therapeutic regimens in the treatment of patients with urea cycle enzymopathies.

A new use is the formulation of sodium benzoate into plastics such as polypropylene, to improve strength and clarity (BFGoodrich Kalama Inc., 1999). Sodium benzoate is used as a stabilizer in photographi baths/processing (BUA, 1995).

Many soft drinks use sodium benzoate as an ingredient. It is a preservative found in a variety of processed foods. Oddly enough, it's also found in antifreeze, because of its anti-corrosive properties. When sodium benzoate combines with ascorbic acid (vitamin c), the result is benzene, and benzene is a dangerous carcinogen.

In short-term studies with rats, disorders of the central nervous system as well as histopathological changes in the brain were seen after feeding high doses of (benzoic acid/sodium benzoate) (1800 mg/kg body weight) over 5–10 days. Other effects included reduced weight gain, changes in organ weights, changes in serum parameters, or histopathological changes in the liver. In a 90-day study with rats dosed with 0, 1, 2, 4, or 8% sodium benzoate via diet, the mortality in the highest dose group (approx. 6290 mg/kg body weight per day) was about 50%. Other effects in this group included a reduced weight gain, increased relative weights of liver and kidneys, and pathological changes (not further specified) in these organs (Deuel et al., 1954).

The information concerning long-term oral exposure of experimental animals to benzoic acid is very limited..In general, the database for benzoic acid and sodium benzoate is limited, and there are no studies available performed according to current guidelines. In addition, the documentation in most cases is limited.

In view of the above cited literatures, the goal of this study was to explore the effect of oral exposure of feed additives sodium benzoate on mood – like behavior in the form of anxiety, depression and social interaction in rats.

2. Materials and methods:

2.1. Animals and housing:

Forty five male albino rats of Wistar strain (80-100 g.) were used in this study. The animals were obtained from Laboratory Animals Unit at Faculty of Veterinary Medicine, Cairo University. They were housed in polypropylene cages with stainless steel wire lids (bedded with wood shavings) and maintained on a standard

Laboratory feed diet throughout the course of the study. Animals had free access to feed and water and housed at a room temperature of 20-22°c, 60% humidity on a 12h light: dark cycle. All efforts were made to minimize the numbers of animals and their suffering in this study through following the

guidelines released by Cairo University Policy on Animal Care and Use.

2.2. Administration of Sodium Benzoate:

Sodium Benzoate (**E**211) was obtained from Sigma Chemical Company (Sigma, Aldrich, USA) and dissolved in tap drinking water at a different concentrations; namely 0%, 0.5 %(low dose) and, 2% (high dose) (Toth 1984)

Forty five male rats were divided into 3 groups (15 rats / group). The experimental design was shown in table (1)

Table (1): The experimental design

Group	Supplemental material.	Dose	Route of administration.	Period of experiment.
Group(C) - control.	Distilled water	0%.	Orally in drinking water.	
Group(L) - Low dose	Sodium Benzoate.	0.5%	Orally in drinking water.	12 weeks
Group (H) - High dose	Sodium Benzoate.	2%	Orally in drinking water.	

2.3. Behavioral assessment:

All behaviors were scored by a single trained observer unfamiliar with treated animals. Hand operated counters and stop watches were used to score animals' behavior.

2.3.1. Anxiety measurements:

The elevated plus-maze was used for testing of anxiety and emotionality. The degree of avoidance of the open arms of the maze has been considered as a measure of strength of fear drive (Trullas and Skolnick, 1993). The apparatus consists of 4 crossed arms, two open arms (50 x 10 x 30 cm) and two closed arms (50 x 10 x 30 cm). The maze was elevated 65 cm above the floor. The rat was placed in the centre of the maze and the number of entries in open and closed arms, respectively, as well as the time the animal spent in the open and enclosed arms during a period of 5 min test session was recorded (Kierstin, 2003; Walf and Frye, 2007). After each trial the maze was wiped out with a cloth dipped in 70% ethyl alcohol and allowed to dry.

2.3.2. Fear and emotional assessment:

The light-dark test has been used to assess the anxiogenic and fear effects of multiple classes of drugs (Jonkman et al., 2005; Kliethermes, 2005). The light-dark box apparatus consisted of a light, open topped, opaque, Plexiglas box connected to a dark, closed topped, Plexiglas box, each compartment measuring (30 x 40 x 40 cm). The boxes were connected by a small opening that allows the rat to cross between chambers. The rat was placed in the light box, allowed to move freely between the chambers, and its location was recorded for 5 min. The time spent on the light side of this apparatus during the 5 min test session compared to the dark side was recorded and used as an index for anti-anxiety behavior (Hascoet et al., 2001; Walf and Frye, 2005). Light box entry was defined as the rat having all four paws into the light box.

2.3.3. Depression – like behavior measurements:

The forced swim test was used for measuring depression – like behavior. Rats were tested in the forced swim test as previously described by Frye and Walf (2002). Rats were placed in cylindrical container (50 x 20 cm) filled with 30 cm of 22°C water. The water level does not allow the rat to rest on its tail, or escape the cylinder by climbing out. The rat was placed in the water for 6 min. The time spent floating (represented immobility) was scored during the last 3 min. The time spent immobile is considered as an index of depression-like behavior in rodents (Sanchez and Meier, 1997).

2.3.4. Social interaction test:

On the day of the experiment, animals were socially isolated in plastic cages measuring (43 x 28 x 15 cm) for 3.5 h prior to the experiment. The task was conducted by placing two animals belonging to the same experimental group, but from different cages, into the test cage for a 15-min period. Tested pairs did not differ in body weight by more than 15 g. The social behavior was assessed for a pair of animals (Schneider and Przewlocki, 2005). The total time spent in social behavior and the numbers of social contacts were measured (Niesink and Van Ree, 1989).

2.4. Statistical analysis:

Data for elevated plus-maze, dark-light transition, depression as well as social interaction tests were analyzed by analyses of variance (AVOVA), using the general linear models procedure in SPSS® statistical software (SPSS, 2006).

3. Results:

3.1. Elevated plus maze test (EPM):

The effect of sodium benzoate on measurements of elevated plus maze was demonstrated in Table 2. Number of entries in closed arms and open arms as well as time spent in the maze was analyzed. Animals under sodium benzoate effects significantly ($p<0.05$) diminished the numbers of entries in the open arms of the maze, accompanied with significant increase of this measure in the closed arms. Concerning time spent in the open arms, exposed individuals showed the shortest time spent in open arm.

3.2. The light-dark test:

As seen in table (3), oral administration of sodium benzoate, caused significant adverse effect on fear and anxiety. Statistical analysis showed that time spent by

sodium benzoate-treated rats in the light compartment was significantly (p < 0.001) diminished, while increased in the dark compartment compared to control counterparts.

3.3. Forced swim test:

Table (4) illustrated the immobility time during forced swim test. A significant increase (p < 0.001) in

the immobility time in rats treated with high doses of sodium benzoate compared to control rats.

3.4. Social interaction test:

As shown in table (5), exposure to sodium benzoate significantly (p < 0.001) affected social interaction parameters as indicated by reduced time engaged in social behavior with decreased numbers of social contacts as well.

Table 2. Effect of Sodium benzoate on anxiety and emotionality of rats during the elevated plus maze test.

	Experimental Groups		
	(C) Group	(L) Group	(H) Group
No. of entries (open arm)	5.47 ± 1.81^a	2.82 ± 1.07^b	1.76 ± 1.30^c
No. of entries (closed arm)	2.47 ± 1.07^a	3.53 ± 1.70^b	5.76 ± 1.97^c
Time spent (open arm) (s)	111.47 ± 20.66^a	77.65 ± 20.99^b	30.24 ± 16.89^c
Time spent (closed arm) (s)	80.12 ± 35.23^a	167.35 ± 30.86^b	201.12 ± 34.66^c

(C) Group: Animals received plain water without any treatment and served as a control.
(L) Group: Animals received low doses of sodium benzoate..
(H) Group: Animals received high doses of. sodium benzoate.
[a-c]Values within row with unlike superscripts differ significantly (**p<0.05**), according to ANOVA. Values are expressed as mean ±SEM, n = 15 in each group.

Table 3. Effect of Sodium benzoate on anxiety and emotionality of rats during the Dark-light transition test

	Experimental Groups		
	(C) Group	(L) Group	(H) Group
Time spent (light compartment) (s)	225.40 ± 37.68^a	169.60 ± 54.07^b	110.54 ± 45.50^c
Time spent (dark compartment) (s)	72.47 ± 37.36^a	129.13 ± 52.22^b	195.54 ± 37.81^c

(C) Group: Animals received plain water without any treatment and served as a control.
(L) Group: Animals received low doses of sodium benzoate..
(H) Group: Animals received high doses of sodium benzoate.
[a-c]Values within row with unlike superscripts differ significantly (**p<0.05**), according to ANOVA. Values are expressed as mean ±SEM, n = 15 in each group.

Table 4. Effect of sodium benzoate on depression – like behavior of rats during the forced swim test.

	Experimental Groups		
	(C) Group	(L) Group	(H) Group
Immobility time (s)	33.90 ± 9.36^a	40.17 ± 11.70^a	85.07 ± 14.92^b

(C) Group: Animals received plain water without any treatment and served as a control.
(L) Group: Animals received low doses of sodium benzoate..
(H) Group: Animals received high doses of sodium benzoate.[a-c]Values within row with unlike superscripts differ significantly (**p<0.05**), according to ANOVA. Values are expressed as mean ±SEM, n = 15 in each group.

Table 5. Effect of sodium benzoate on the behaviour of rats in the social interaction test.

	Experimental Groups		
	(C) Group	(L) Group	(H) Group
Time spent in social interaction (s)	304.44 ± 35.70^a	199.33 ± 20.17^b	180.22 ± 27.10^c
No. of social contacts	60.44 ± 9.25^a	40.11 ± 10.54^b	17.89 ± 6.94^c

(C) Group: Animals received plain water without any treatment and served as a control.
(L) Group: Animals received low doses of sodium benzoate.
(H) Group: Animals received high doses of sodium benzoate.

[a-c]Values within row with unlike superscripts differ significantly (***p*<0.05**), according to ANOVA. Values are expressed as mean ±SEM, n = 15 in each group.

4. Discussion:

In this work, the effect of sodium benzoate in drinking water on the following parameters, anxiety and fear, depression as well as anti- social behavior in male rats, were recorded. Anxiety in rats can be measured by behavioral reactivity to non-social or social stressors (Kim et al., 2004). These behaviors were compared by performing the elevated plus maze and the light-dark transition tests (non-social) as well as social interaction test (social). With regard to the present study, it is important to note that most of the behavioural models cited above have mainly been used in the studies on the neurobiological mechanisms implicated in the production of fear and anxiety elicited in animals exposed to aversive situations (Rodgers, 1997; Rodgers and Dalvi, 1997; Menard and Treit, 1999).

Our results, revealed that, sodium benzoate caused a significant increase in the anxiety levels of rats. Regarding anxiety measurement in elevated plus maze, decreased visits for open arms of elevated plus maze were recorded in our study after exposure to sodium benzoate. However, a dose-dependent effect of sodium benzoate on time spent in the open arms was evidently shown, where the less time was observed with high dose-administered rats indicating that they avoid this aversive region of the maze. Therefore, time elapsed in the open arms might be considered as the more sensitive index for anxiety than number of visits.

The light-dark test has been used to assess the anxiogenic effects of multiple classes of drugs (Jonkman et al., 2005; Kliethermes, 2005). The measurements of dark-light transition test also support the former observation of sodium benzoate - induced anxiety-like responses, where marked diminution in time spent in light compartment was noticed with sodium benzoate -exposed rats. These results are consistent with former study with rats (Tarriq et al., 2004). Also, Collaborative Health and the Environment (2008) confirmed that combinations of some synthetic food colors and / or the preservative sodium benzoate have been associated with increased irritability, restlessness and sleep disturbance in some children.

Regarding depression- like behavior, the forced swim test measures behavioural despair in rodents (Raghavendra et al., 2000). Our results, of forced swim task revealed that sodium benzoate exposed animals exhibited higher immobility; an index of depression-like behaviour (Sanchez and Meier, 1997), as a response for increased levels of stress reaction. Further support derived from earlier human

study for Southwick et al. (2005), where elevated cortisol in response to chronic stress was associated with increased manifestations of depression. Experimental evidence indicates that unusual reactions to food additives (Tartrazine and benzoates) involving mainly the central nervous system (headache, migraine, over-activity, learning difficulties and depression) (Novembre et al. 1992). In addition, in short term studies with rats, disorders of the central nervous system (benzoic acid / sodium benzoate) as well as histopathological changes in the brain were seen after feeding high doses over 5- 10 days (BFGoodrich Kalama Inc., 1999).

Since many social disorder models in rodents are linked to human social deficits syndrome, social interaction test has been implemented in the current study. Aberrant social behaviours or low levels of social interaction are symptoms of several psychiatric disorders, including anxiety, depression and social phobias (Crawley, 2007). Artificial or synthetic food colors and additives are ubiquitous in the food supply and have long been suspected of causing conduct disorders (weiss et al., 1980, Schab and Trinh 2004).A profound reduction in time engaged in social interaction was observed in this work accompanied with decreased frequency of social contacts following exposure to sodium benzoate. The most interesting finding was the dose-related reducing effect on bouts of social contacts. These antisocial-related findings confirm our formerly reported results for increased hyperactivity, anxiety and depression in sodium benzoate-treated rats. Previous and recent carefully conducted double – blind human studies have confirmed that artificial food colorings such as sun yellow, tartrazine, carmoisine and ponceau as well as the preservative sodium benzoate, can cause conduct disorders (weiss et al., 1980; Rowe and Rowe 1994). Piper (1999) claims that sodium benzoate by itself can damage and inactivate vital parts of DNA in a cell's mitochondria. Mitochondria consume oxygen to generate ATP, the body's energy currency. If they are damaged due to disease, the cell malfunctions and may enter apoptosis (Martin, 2007 and Piper, 1999).

Our results strongly provides sufficient scientific evidence that a causal link truly exists between sodium benzoate and inflection of anxiety, depression-like behaviors and anti- social behavior in rats and points to the hazardous impact of sodium benzoate on public health.

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