

Aspartame Consumption Increases Glutathione Peroxidase Level and Depression- Like Behavior in Rats

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Abstract: Aspartame is a worldwide used artificial sweetener and is consumed by millions of adults and children as part of their diet. The connection between aspartame ingestion and depression has been studied with contradictory results. We hypothesized that this correlation might be explained by high levels of oxidative stress. We wanted to examine the biochemical effects of consuming higher or lower doses of aspartame on the antioxidant enzyme- glutathione peroxidase (GPX) and on the depression-like behaviors of rats in the forced swim test model. 40 lab rats were divided into 4 groups; two groups were treated by gavage with either 75 mg/kg/day of aspartame or 125 mg/kg/day of aspartame. The control group received a gavage with vehicle (water). The naïve group received no experimental intervention. Our statistical analysis revealed that the rats from the control group and the naïve group presented a significantly lower level of GPX compared to the groups that received 75mg/kg/day or the group that received the maximum dosage of 125mg/kg/day aspartame. Furthermore, a shorter duration of the immobility was reported in the control and the naïve groups when compared to the groups which received any of the two dosages of aspartame. Therefore, the results presented by our study suggest that aspartame consumption (in both high and low dosages) increases both the oxidative stress and the depression-like symptoms in the forced swim model. In addition, an aspartame dose-response was not found for either of our two variables: oxidative stress or depression. In conclusion, the daily consumption of aspartame for 4 weeks, in both high dosage and low dosage, had a negative impact on both oxidative stress level and the frequency of depression- like behaviors of the animals in the forced swim test. These results suggest that the correlation between aspartame ingestion and depression might be explained by oxidative stress levels.

Keywords: aspartame, glutathione peroxidase, oxidative stress, depression, artificial sweetener.

1. Introduction

Aspartame is a worldwide used artificial sweetener and is found in numerous food products. Extensive surveys show that a high percent of adults and children consume aspartame as part of their daily diets [1-3]. The history of this artificial sweetener shows that aspartame was first approved in the United States by the Food and Drug Administration in 1981; however this approval was for limited use only. It was approved as a general sweetener later on in 1996. Furthermore, a maximal recommended intake still exists today. Specifically, an acceptable daily intake is considered to be around 40 mg/kg body weight/day and is recommended by The World Health Organization and the majority of other food regulatory authorities [4]. Any intake above that dosage is considered an excessive intake of aspartame [4]. In addition, the recommended dosage slightly varies from country to country. For example, the US FDA considers a dosage of aspartame at 50 mg/kg bodyweight/day as safe. However, the before mentioned dosages are recommended as safe for healthy individuals. For example, specialists recommend that individuals with phenylketonuria should not consume aspartame at all considering that phenylalanine is a known metabolite.

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The biochemical mechanism behind this recommendation is that aspartame once ingested is metabolized to aspartic acid, phenylalanine, and methanol [5]. It is well demonstrated that phenylalanine is involved in neurotransmitter regulation [6]. Furthermore, the aspartic acid is also an important excitatory neurotransmitter [6]. Therefore, the ingestion of large quantities of aspartame can lead to various dysfunctions in certain neurotransmitter regulation which can result in the observed neurobehavioral disorders. Studies clearly show that following aspartame consumption individuals present substantial increases in phenylalanine and aspartic acid [7]. Furthermore, as a result of that increase, the same individuals also present reduced dopamine and serotonin production [7]. Therefore, the results reported by these authors suggest that aspartame metabolites seem to be responsible for the neurobehavioral alteration observed after the ingestion of high doses of aspartame [8].

In addition, beside its direct influence on the neurotransmitters, aspartame also has a negative effect on the blood–brain barrier. This negative effect refers to the fact that following aspartame ingestion a high permeability and altered concentrations of catecholamine's level in the brain, such as dopamine, were reported. Therefore, these altered processes may be the possible biochemical mechanism behind aspartame consumption's role in the pathogenesis of certain psychiatric disturbances. The main arguments against these findings are made by authors who bring to account that very high concentrations of aspartame are needed in order for these detrimental effects to be observed and measured [9].

Therefore, despite its common usage and its well documented safety at low daily dosages, aspartame still continues to be one of the most polemical food additives of our time [10]. A consensus in the science community has not yet been reached, with some studies reporting that aspartame metabolites are responsible for adverse effects, such as headache, compromised memory, mood changes, and depression; other experiments failing to identify any adverse effects of any kind.

Specific to depressive disorders, the connection between aspartame ingestion and depression has been studied with contradictory results. For example, a well-known study with a sample of 40 individuals with depression and 40 healthy controls, who received a high dose of aspartame (30 mg/kg body weight/day), had to be stopped by the institutional review board after only 13 of the initial participants completed the study, due to the severity of depressive symptoms reported by the vast majority of the individuals [11]. Contradictory to these results, a study on 133 women with normal body weight and 53 overweight women, who consumed aspartame or sucrose-sweetened beverages over 4 weeks, reported no differences in depressive symptoms between the women who drank aspartame-sweetened and the women who consumed sucrose-sweetened sodas [12,13].

Furthermore, the literature shows that after chronic ingestion of aspartame, detectable levels methanol are found to circulate in the blood [14]. In addition, methanol and its metabolites is known to be responsible for decreasing the levels of various antioxidant enzymes, such as glutathione peroxidase (GPX) and therefore stimulate the production of oxidative stress in the cortex. Moreover, other studies also confirm the existence of toxic metabolites in the brain following aspartame ingestion that eventually lead to high levels of oxidative stress [14].

Therefore, given the still contradictory results available in the literature regarding the connection between depression and aspartame, and the well documented correlation between aspartame administration and elevated levels of oxidative stress, the objective of this study was to examine the biochemical and neurobehavioral effects of consuming higher (125 mg/kg body weight/day) or lower (75 mg/kg body weight/day) doses of aspartame on the brain levels of oxidative stress (measured by the level of the antioxidant enzyme glutathione peroxidase- GPX) and on the depression- like behaviors of rats in the forced swim test model.

2. Materials and methods

Our experimental design was constructed so that 40 Sprague-Dawley rats were split into 4 groups, three of which were treated by gavage with either a low dose of 75 mg/kg/day of aspartame or a high dose of 125 mg/kg/day of aspartame. The control group received a gavage with vehicle (water). The

naive group received no experimental intervention. The rats in the two aspartame groups received the gavage treatment daily for 4 weeks.

After the 4 weeks of the initial phase of our experiment, the animals from all 4 experimental groups were tested in the forced swim model in order to measure and quantify the depression-like behaviors. Each rat was positioned individually in glass cylinders (with standard measures used in this model) containing 30 cm of water. Later on, after they spent 15 minutes in the water the rats were returned to their cages. Next, after a 24 hour break, they were replaced in the water. This was the moment when a blinded researcher measured with a stopwatch the duration of immobility displayed by the rats. The rats were considered immobile when they remained floating without resistance in the 30 cm of water.

The biochemical measure of GPX was conducted according to a previous validated method [15] and with the help of a kit from Sigma Aldrich. The GPX activity is expressed in our data as nmol/mg protein.

3. Results and discussions

After we created our data base, we ran an One Way ANOVA analysis using SPSS and we found that there was significant difference between our four experimental groups regarding the glutathione peroxidase assay, $F(3, 36) = 59.355$, $p < 0.001$.

Besides the ANOVA we also ran a post hoc Fisher's Least Significant Difference (LSD) comparisons between the four groups. The results showed significant differences between the naive and the group that received the gavage 75 mg/kg/day of aspartame ($p < 0.001$) in regards to glutathione peroxidase assay. Furthermore, also significant differences were found between the naive group and the group which received the gavage 125 mg/kg of aspartame ($p < 0.001$), the control group and the group which received the gavage 75 mg/kg/day of aspartame ($p < 0.001$), control group and the group which received the gavage with 125 mg/kg/day of aspartame ($p < 0.001$). However, our post hoc analysis found no significant difference regarding glutathione peroxidase assay between the naive group and the control group ($p = 0.302$). No significant difference were also found between the group that received the gavage with 75 mg/kg/day and the group that received the gavage with 125 mg/kg/day ($p = 0.135$). The mean and the standard deviation for every experimental group can be seen in Table 1.

Table 1. Glutathione peroxidase assay for every experimental group (mean and SD) measured in nmol/mg protein

Experimental group	Glutathione peroxidase assay (mean)	Glutathione peroxidase assay (SD)
Naive group	41.2	2.699
Control group	39.9	2.424
75 mg/kg/day	29.9	2.806
125 mg/kg/day	28	3.126

Furthermore, there was also a significant difference between our four groups of rats regarding the time that the animals spent being immobile in the water, $F(3, 36) = 31.878$, $p < 0.001$.

As in the case of our previous variable, besides the ANOVA we also performed a post hoc Fisher's Least Significant Difference (LSD) comparisons between our experimental groups. The results presented significant differences between the naive and the group that received the gavage 75 mg/kg/day of aspartame ($p < 0.001$) in regards to the total time of immobility. In addition, also significant differences were found between the naive group and the group which received the gavage 125 mg/kg of aspartame ($p < 0.001$), the control group and the group which received the gavage 75 mg/kg/day of aspartame ($p < 0.001$), control group and the group which received the gavage with 125 mg/kg/day of aspartame ($p < 0.001$). On the other hand, our post hoc analysis found that there were no significant difference regarding the immobility time between the naive group and the control group ($p = 0.06$). In addition, no significant difference were also found between the group that received the gavage with 75 mg/kg/day and the group that received the gavage with 125 mg/kg/day ($p = 0.347$). The mean and the

standard deviation of the duration of immobility for every experimental group can be observed in table 2.

Table 2. Duration of immobility for every experimental group (mean and SD) measured in seconds

Experimental group	Duration of immobility (mean)	Duration of immobility (SD)
Naive group	81.1	7.838
Control group	88.2	11.253
75 mg/kg/day	106.3	6.617
125 mg/kg/day	109.6	2.836

When we designed the present study our main focus was to discover if administrating aspartame, in various dosages to rats, will increase the oxidative stress level in the brain of our rats. Furthermore, we wanted to discover if this increase in oxidative stress will be accompanied by an escalation of depression- like behaviors. Our statistical analysis revealed that aspartame ingestion, in both high and low doses increases the level of oxidative stress, by decreasing the measured level of GPX in the cortex of our rats. Our data showed that rats from both aspartame groups had a significant lower level of the antioxidant enzyme-GPX in the brain compared to both the naïve and the control group. In addition, regarding the depression, the results retrieved by our statistical analyses demonstrated that the rats from the control group and the naïve group presented a significantly shorter duration of the immobility compared to the experimental groups that received a gavage with 75mg/kg/day or the group that received the gavage with 125mg/kg/day of aspartame. Therefore, we can deduce from these results that the aspartame consumption (in both high and low dosages) significantly increases oxidative stress, which is also associated with an escalation in the depression-like symptoms in the forced swim model of depression.

The second goal of our study was to determine if a low dose of aspartame will differently influence the level of the antioxidant enzyme we measured (GPX) and the depression- like behavior of the rats compared to a high dose of aspartame. The results of our analyses demonstrated that such a dose-response of aspartame does not exist. A high dose of aspartame will produce similar effects on the oxidative stress level to a lower dose. Although the rats in the 125mg/kg/day group had lower level of GPX in their brain compared to the 75mg/kg/day group, these differences were not statistically significant. The same results were obtained regarding the effect of different doses of aspartame on the depression like behavior: no statistically significant difference was found.

In consensus with our study, other literature reports on the connection of aspartame and oxidative stress also demonstrated that aspartame consumption influences the level of oxidative stress in the brain. For example, the aspartame intake has been reported to be responsible for decreasing the level of two antioxidant enzymes: superoxide dismutase and glutathione peroxidase [16]. Moreover, other studies have reported that the daily consumption of aspartame for 4 weeks lead to a significant decrease in the GPX levels in the cerebral cortex. Furthermore, these observed effects were accompanied by a significant increase in various markers of oxidative stress, such as malondialdehyde (MDA). In addition a significant decrease in superoxide dismutase (SOD) activity was also reported [17]. All of these reported results are in concordance with our findings. Additionally, according to these studies [16,17], the decrease in GPX and SOD content and increase in MDA activity persisted until after 42 days of aspartame chronic consumption. Therefore, it is important to be mentioned that aspartame-induced oxidative stress may persist for longer periods even after the daily ingestion has stopped. This persistence of high levels of oxidative stress might be explained by the possible accumulation of methanol and its metabolite that occurs even if only the acceptable daily intake dose is consumed.

Furthermore, an involvement of aspartame in the pathophysiology of depression is likely and has been previously demonstrated in the literature. For example, in a study on mice, the reported results showed that the levels of norepinephrine and dopamine were increased in several brains regions after a

single dose of aspartame [18]. The increased levels were observed in many regions of the cortex, but mostly in the hypothalamus, in which the level of norepinephrine is particularly high. Interestingly, only increased activities of adrenergic neurotransmitters were reported and no alteration of serotonin was observed by the authors [19]. Although the levels of neurotransmitters are largely dependent on the availability of other precursor amino acids, they are also adjustable to homeostatic adaptations that occur in the brain [20,21].

The results reported here accompanied by those found in the literature suggest that aspartame ingestion may produce alterations in oxidative stress levels in various brain regions. However, some studies found that alterations of the serotonin levels were found in the hypothalamus, the area that is known to be extremely important for neuroendocrine control [22]. Other experiments reported that feeding rats a diet with only 5% aspartame caused a significant decrease in whole-brain serotonin receptors levels, when compared to the control group [23].

Therefore, the aspartame- induced elevation of depression-like behavior observed in our experiment is explained by the levels of oxidative stress that we found to be elevated in the brain of the rats. However, our results might also be related with the increased levels of norepinephrine and dopamine, the transmitters that have phenylalanine and tyrosine as their precursors. These effects should be greater in the hypothalamus compared to other regions of the brain. Furthermore, repeated treatment with aspartame was also shown to increase concentrations of catecholamine neurotransmitters, increase observed in various brain regions [24].

These findings from animal models can lead to a better understanding of the pathology of human depressive disorders. This is an important aspect, given that an individual's depressive symptoms may impact, among others, decision making, and both long-term and short term memory [25]. Therefore, certain alterations in the human brain may be influenced by the consumption of aspartame. These changes refer to an observed increase in the concentrations of cerebral phenylalanine and certain alterations in brain neurotransmitters levels.

Therefore, any possible influence that aspartame may have on serotonin may be crucial and should be investigated, given that this neurotransmitter is important in handling the emotional information in humans [26]. In addition, serotonin pathways also regulate emotional control in limbic structures such as cingulate gyrus, the amygdala and hippocampus [27]. Serotonin receptors pathways in the prefrontal lobe and amygdala contribute to the sensitivity of the emotional processing connections [28]. Therefore, certain disorders in the activity of the serotonin receptors may contribute to alterations in the processing of certain emotions, leading individuals to mood swings and depression [29]. On the other hand, a low level of serotonin may negatively influence the mood of an individual by impairing neural activity in the ventral anterior cingulate, caudate nucleus and orbitofrontal cortex [30].

Although studies on the possible negative effect of aspartame on depression in human subjects are limited, some authors have succeeded in demonstrating this effect. For example, in a study on a sample of university students, the reported results showed that students who consumed a high (25 mg/kg body weight/day) dose of aspartame presented more frequent depression episodes compared to students who consumed a lower (10 mg/kg bodyweight/day) dose of aspartame [31]. Other study found that individuals who already had a mood disorder were more sensitive to aspartame consumption compared to healthy controls [11]. However, not every study that investigated the effect of both high and low dosages of aspartame on depression had significant results. Therefore, the possible negative effects of aspartame on depressive disorders are still controversial.

4. Conclusions

In our sample of rats, the daily consumption of aspartame for 4 weeks, in both high dosage and low dosage, had a negative impact on the level of oxidative stress that was measured in their brain. The rats in the control and the naïve group had a statistically significant higher level of GPX compared to both high dose and low dose of aspartame groups. As expected, no significant difference was found between the naïve group and the control group. Furthermore, no significant difference was found

between the group of rats which received 125mg/kg/day of aspartame and the group of rats which received 75mg/kg/day of aspartame, suggesting that a relatively low dose of aspartame is sufficient to increase the level of oxidative stress of the animals in our experiment. Moreover, the same results were found regarding the connection between aspartame ingestion and depression- like behavior in the forced swim test. All these results suggest that oxidative stress might explain the elevation observed in depression- like behaviors after aspartame consumption. Unfortunately, findings from animal experiments should not be directly extrapolated to humans. For instance, the majority of animal studies on the aspartame-depression connection have used very high doses of aspartame that are not relevant for day by day human consumption of this artificial sweetener. Therefore, definite conclusions should not be drawn completely from this paper without proper future human studies. Instead, our findings should be followed by extensive work on the possible negative effect that aspartame may have on general human cognition.

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Manuscript received: 6.05.2020