OBJECTIVE: Sulfites are commonly used as preservatives in the foods and pharmaceutical products. Despite their worldwide use, increasing evidence suggests their toxicity on several organs and tissues. Yet, we do not know whether sulfites have toxic effects on the auditory system (tract). Therefore, we aimed to investigate the probable hazardous effects of sulfite on hearing function of rats.

MATERIAL AND METHODS: 40 male Wistar albino rats were divided into four equal groups: 1- Control (C) 2- Sulfite treated (S) 3- Vitamin E treated (E) 4- Vitamin E + Sulfite treated (ES). Auditory Brainstem Response data were collected from all of the groups at the beginning of the study as baseline and at the end of 8 week after the experimental period ended.

RESULTS: Sulfite treatment created a significant elevation on hearing thresholds, wave latencies (except for Wave I. absolute latency) and interpeak interval of rats. Further, these ototoxic effects of sulfite could significantly be prevented by vitamin E administration.

CONCLUSION: These findings designated a preponderance of toxicity in the auditory neural pathways with a probable mechanism of free radical production. To our knowledge, this is the first study in the literature demonstrating the toxic effects of sulfites on hearing function of rats.
Humans are exposed to both endogenous and exogenous sulfites. Considerable amount of sulfites is generated endogenously by the metabolism of sulfur-containing amino acids such as methionine and cysteine. Exogenous sources of sulfites include foods and beverages, ambient air, and pharmaceutical products. Sulfites are widely used in food processing to sanitize fermentation equipment and food containers, to reduce or prevent microbial spoilage of foods, to selectively inhibit undesirable microorganisms in fermentation industries and to prevent oxidative discoloration and non-enzymatic browning during preparation, distribution and storage of food and beverages. Sulfite is also used as a stabilizer in many drugs administered to patients. Five sulfite salts including sodium metabisulfite (Na2S2O5), potassium metabisulfite (K2S2O5), sodium bisulfite (NaHSO3), potassium sulfite (K2SO3), and sodium sulfate (Na2SO3) are commonly used for these purposes in food and pharmaceutical preparations. It is reported that ingested sulfite enters into the systemic circulation via gastrointestinal absorption and distributed essentially to all body tissues including the brain. Both an endogenously generated and an exogenously intake of sulfite must be detoxified because it can react with a variety of humoral and cellular components and can cause toxicity. For this purpose, mammalian tissues contain sulfite oxidase (SOX), which catalyzes the oxidative detoxification of sulfite to sulfate (SO42-) if there is a deficiency of sulfite oxidase or exposure to excessive sulfite, the sulfite undergoes one electron oxidation reactions which is catalyzed by peroxidases to form sulfur trioxide anion radical (SO3-). The sulfite radical can further react with molecular oxygen forming sulfite peroxyl (SO3OO.) radical and sulfate (SO42-) radical. There are substantial data from in vitro studies that these sulfite radicals have the ability to react with several molecules of biological importance, including DNA. Moreover, central nervous system effects have recently been demonstrated following sulfite exposure in rodents. Increased latencies in both visual and somatosensory evoked potentials have been reported by our group following sulfite inhalation and ingestion. It is also demonstrated that sulfite ingestion induced impairment of active avoidance learning in rats. Physiological importance of this detoxification is also seen in humans by consequences of SOX deficiency, which is a genetically inherited disease. The associated severe neurological dysfunction is characterized by dislocation of ocular lenses, mental retardation, and attenuated growth of the brain suggests that neuronal cells are susceptible to sulfite toxicity. Activity of SOX in mammalian tissues exhibits a large distribution, and its activity in tissues shows significant differences even in the same species. For instance, liver, kidney and heart tissues have high SOX activities, whereas brain, spleen and testis have very low activities. Among cells, therefore, neuronal cells may be especially vulnerable to sulfite because of their low SOX activity. Although reactive oxygen species (ROS) were implicated as increased toxicity of sulfite, exact mechanism was not elucidated. Moreover, oxidative stress associated with ROS caused by sodium sulfite can be controlled to a certain degree by antioxidants such as vitamin E with free radical scavenging action. Oxidative stress and free radicals have also been implicated in a wide variety of pathological conditions in the cochlea and auditory pathways such as noise exposure, use of aminoglycoside antibiotics, use of anti-neoplastic agent, cis-platin and presbyacusis. Despite these data on various types of free radical based neurotoxicity, we do not know effects of sulfites on hearing function of mammals. Therefore, in this study we aimed to investigate whether sulfite ingestion had harmful effects on auditory pathways of rats via the evaluation of Auditory Brainstem Responses (ABR).

MATERIALS AND METHODS

Preparation of animals:
Forty male albino Wistar rats, weighing 250-300 g, were used throughout the study. They were provided from Akdeniz University animal breeding colony and housed in stainless steel cages in groups of 5 rats per
cage. Animals were maintained at 12 h light-dark cycles with a constant temperature of 24 ± 2ºC and 50 ± 5 % humidity at all times. Rats were divided into four groups of 10 animals each: 1) Control (C); the rats were fed with standard laboratory chow and tap water ad libitum. 2) Sulfite (S); the rats were treated with sulfite in the form of sodium metabisulfite ($Na_2S_2O_5$) at a dose of 25 mg/kg/day by gastric gavage (Merck, Darmstadt, Germany). 3) Vitamin E (E); rats received 50 mg/kg/day vitamin E by gastric gavage. 4) Sulfite + vitamin E (SE); rats in this group received sodium metabisulfite (25 mg/kg/day) and 50 mg/kg/day of vitamin E by gastric gavage. Sodium metabisulfite suspended in distilled water and Vitamin E dissolved in olive oil. Olive oil was also administered as a vehicle to the groups not receiving the vitamin E doses. All these treatments were continued for 8 weeks. The daily food and water consumption of rats in all experimental groups were measured during the feeding period. The body weights were recorded weekly.

Anesthesia:

Rats in the all experimental groups were anesthetized with 12 mg/kg xylazine and 65 mg/kg ketamine in order to record baseline ABR data at the beginning of the study and for final ABR measurements at the end of 8 weeks. The animals were then killed by exsanguinations.

ABR Recordings:

The animals were examined under anesthesia and confirmed to have normal external auditory canal and tympanic membranes before the baseline and final audiometric measurements. During the tests, room temperature was maintained at 210 C and the rats under general anesthesia were warmed up by an electrical heater to stabilize their normal body temperatures. Oral temperatures of the animals were stabilized at 37.5 to 390 C. Baseline ABR data of the animals in all groups were recorded at the beginning of the study. Repeated ABR measurements were performed at the end of 8 weeks.

Auditory Brainstem Responses were recorded in a sound proof cabinet as previously described. Briefly, a reference silver needle electrode affixed to the vertex, an active recording silver needle electrode was attached to the ipsilateral mastoid. A disc ground electrode was placed on the tail. Medelec Audioscan Portable Evoked Response Audiometers usually measure fast, middle and late latencies of ABR in patients. Since the measurement of late latency responses (300 ms) require awakeness, only the fast latency technique (1-10 ms) which are normally used for electrophysiological evaluations were recorded in the anaesthetized rats. A special probe (Medelec ear tips Neonatal Part No: 51019) was introduced into the external auditory canal then sound stimuli were delivered via Medelec intra-auricular headset (S 51013) to animals.

An alternating click stimulus with 10/sec repetition rate was used to elicit action potentials in the auditory system. Each waveform was obtained from 1024 sweeps. Click stimuli were calibrated with a calibrated B&K precision sound level meter (duration 100 ms, stimulation rate 10/s, and frequency from 0 to 10,150 Hz). The equipment used in our study can produce 0-110 dB nHL stimulus intensity and record waveforms with a 300Hz- 3KHz band-pass filter. We defined the lowest intensity of click stimuli at which wave V of the ABR could just be detected as the ABR threshold (dB nHL) level in rats. Since the click stimuli between 70 and 90 dB dB nHL are accepted as optimal levels for measurement of the wave I., 70 dB nHL was used as a suprathreshold level in our study. ABR measurements were elicited as waves I., III., V. and I-III, III-V, I-V interpeak intervals (IPI) bilaterally. Complete ABR testing of each animal was lasted approximately 30-35 minutes. Since there was no statistically significant difference among the parameters of right and left ears of rats in each group (data not shown), only the data from right ears were documented.

Statistical Analysis

The statistical analysis of the obtained data was performed by SPSS 15.0 software for Windows. The results are expressed as mean ± SEM. The baseline and posttreatment ABR values within each group were compared by Wilcoxon Signed Ranks Test. The differences in the ABR values among the different
groups were analyzed via Kruskal Wallis one-way analysis of variance on ranks with all pair wise multiple comparisons performed by Mann-Whitney U test. Values of $p<0.05$ was considered as statistically significant.

All experimental protocols conducted on rats were performed in accordance with the standards established by the Institutional Animal Care and Use Committee at Akdeniz University, Medical School.

---

**RESULTS**

**General animal health:**

All the animals were outwardly healthy. Rats in the all experimental groups did not demonstrate abnormal clinical signs of toxicity throughout the study. Treatment groups exhibited similar weight gains and survival.

**ABR Results:**

Overall baseline and final ABR data of the groups are presented in Table 1. There was no statistically significant difference between the groups for any of the baseline values (ABR thresholds, I, III and V. wave latencies and I-III, I-V and III-V IPL) (Figures 1, 2, 3, 4, 5, 6 and 7). When baseline and posttreatment ABR values were compared within each group, the rats in the sulfite treated group (S) demonstrated significant threshold deterioration, latency and IPL prolongations in their posttreatment evaluations ($p<0.05$ for thresholds, $p<0.01$ for all wave latencies and I-III IPL, $p<0.001$ for III-V and I-V IPL respectively) (Figures 1, 3, 4, 5, 6 and 7). Posttreatment I-V IPL value was also significantly prolonged ($p<0.05$) in the Sulfite+Vitamin E treated group (SE) when compared to the baseline (Figure 7). However, I. Wave latencies of the four groups and all threshold, latency and IPL values of the groups C, E

<table>
<thead>
<tr>
<th></th>
<th>Control (C)</th>
<th>Sulfite (S)</th>
<th>Vitamin E (E)</th>
<th>Sulfite + Vitamin E (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline ABR Threshold (dB)</strong></td>
<td>23 ± 1.52</td>
<td>23 ± 1.52</td>
<td>22 ± 2.00</td>
<td>21 ± 1.00</td>
</tr>
<tr>
<td><strong>Final ABR Threshold (dB)</strong></td>
<td>23 ± 1.52</td>
<td>28 ± 1.33</td>
<td>23 ± 3.00</td>
<td>23 ± 1.84</td>
</tr>
<tr>
<td><strong>Baseline I. Wave Latency (ms)</strong></td>
<td>1.28 ± 0.065</td>
<td>1.20 ± 0.058</td>
<td>1.25 ± 0.099</td>
<td>1.21 ± 0.027</td>
</tr>
<tr>
<td><strong>Final I. Wave Latency (ms)</strong></td>
<td>1.15 ± 0.016</td>
<td>1.31 ± 0.082</td>
<td>1.27 ± 0.089</td>
<td>1.27 ± 0.021</td>
</tr>
<tr>
<td><strong>Baseline III. Wave Latency (ms)</strong></td>
<td>2.77 ± 0.040</td>
<td>2.70 ± 0.069</td>
<td>2.78 ± 0.064</td>
<td>2.73 ± 0.11</td>
</tr>
<tr>
<td><strong>Final III. Wave Latency (ms)</strong></td>
<td>2.76 ± 0.030</td>
<td>3.65 ± 0.084</td>
<td>2.85 ± 0.080</td>
<td>2.86 ± 0.022</td>
</tr>
<tr>
<td><strong>Baseline V. Wave Latency (ms)</strong></td>
<td>3.95 ± 0.068</td>
<td>3.82 ± 0.037</td>
<td>3.91 ± 0.088</td>
<td>3.95 ± 0.088</td>
</tr>
<tr>
<td><strong>Final V. Wave Latency (ms)</strong></td>
<td>3.82 ± 0.061</td>
<td>5.62 ± 0.100</td>
<td>4.04 ± 0.105</td>
<td>4.23 ± 0.076</td>
</tr>
<tr>
<td><strong>Baseline I-III IPL Latency (ms)</strong></td>
<td>1.50 ± 0.028</td>
<td>1.50 ± 0.038</td>
<td>1.56 ± 0.045</td>
<td>1.56 ± 0.046</td>
</tr>
<tr>
<td><strong>Final I-III IPL Latency (ms)</strong></td>
<td>1.58 ± 0.030</td>
<td>2.44 ± 0.120</td>
<td>1.57 ± 0.047</td>
<td>1.58 ± 0.027</td>
</tr>
<tr>
<td><strong>Baseline III-V IPL Latency (ms)</strong></td>
<td>1.19 ± 0.055</td>
<td>1.23 ± 0.056</td>
<td>1.23 ± 0.100</td>
<td>1.28 ± 0.044</td>
</tr>
<tr>
<td><strong>Final III-V IPL Latency (ms)</strong></td>
<td>1.14 ± 0.077</td>
<td>1.96 ± 0.093</td>
<td>1.18 ± 0.078</td>
<td>1.34 ± 0.071</td>
</tr>
<tr>
<td><strong>Baseline I-V IPL Latency (ms)</strong></td>
<td>2.60 ± 0.067</td>
<td>2.64 ± 0.066</td>
<td>2.63 ± 0.042</td>
<td>2.59 ± 0.060</td>
</tr>
<tr>
<td><strong>Final I-V IPL Latency (ms)</strong></td>
<td>2.62 ± 0.064</td>
<td>4.40 ± 0.14</td>
<td>2.76 ± 0.086</td>
<td>2.91 ± 0.116</td>
</tr>
</tbody>
</table>

Baseline and final ABR values of all groups are demonstrated. Presented data are mean values ± SEM of each group (n=10). ms: milliseconds; dB : decibel.
Auditory brainstem response disturbances in rats induced by sodium metabisulfite ingestion

**Figure 1:** Pair wise analyses of values between groups as well as the comparison of the baseline and final (posttreatment) measurements within each group for ABR thresholds are demonstrated. The presented data are mean ± SEM of each group (n=10). *; significant difference when compared to the baseline threshold of the group S and the final thresholds of the groups C and SE (p<0.05), #; significant difference when compared to the posttreatment threshold of group E (p<0.01).

**Figure 2:** Pair wise analyses of the values between groups as well as the comparison of the baseline and final values within each group for Wave I latency did not show statistically significant difference. The presented data are mean ± SEM of each group (n=10).

**Figure 3:** Pair wise analyses of values between groups and comparison of the baseline and final measurements within each group for Wave III latencies are demonstrated. The presented data are mean ± SEM of each group (n=10). *; significant difference when compared to the baseline values of group (S) (p<0.01), #; significant difference when compared to final values of the group C, E, and SE (p<0.001), †; significant difference when compared to the final values of group C (p<0.05).

**Figure 4:** Pair wise analyses of values between groups and comparison of the baseline and final measurements within each group for Wave V latency are demonstrated. The presented data are mean ± SEM of each group (n=10). *; significant difference when compared to the baseline values of group S (p<0.01), #; significant difference when compared to the final values of groups C, E and SE (p<0.001), †; significant difference when compared to the final values of group C (p<0.01).

**Figure 5:** Pair wise analyses of values between groups and comparison of the baseline and final measurements within each group for I-III IPL. The presented data are mean ± SEM of each group (n=10). *; significant difference when compared from the baseline values of group (S), final values of the groups C, E, and SE (p<0.01).

**Figure 6:** Pair wise analyses of values between groups and comparison of the baseline and final measurements within each group for III-V IPL. The presented data are mean ± SEM of each group (n=10). *; significant difference when compared to the baseline values of group S and final values of the groups C, E and SE (p<0.001).
and SE (except for I-V IPL for this group) were not significantly different on their posttreatment measurements (Figures 1, 2, 3, 4, 5, 6 and 7). In the comparison of posttreatment ABR values between groups, we found that the thresholds, latencies and IPL values in the group S were significantly higher than the groups C, SE (p<0.05 for thresholds, p<0.001 for III., V. Wave latencies and III-V, I-V IPL, p<0.01 for I-III IPL) and E (p<0.01 for thresholds and I-III IPL, p<0.001 for III. and V. wave latencies and III-V and I-V IPL respectively) (Figure 1, 3, 4, 5, 6 and 7). No statistically significant difference could be observed between the posttreatment threshold values, all latencies and IPL of the groups C and E (Figure 1, 3, 4, 5, 6 and 7). Posttreatment Wave I. latencies between the four groups did not show any significant difference either (Figure 2) but posttreatment Wave III. and V. latencies were significantly higher in the SE group when compared to the posttreatment values of the group C (p<0.05 for III and p<0.01 for V respectively) (Figures 3 and 4).

**DISCUSSION**

Our study demonstrates that sub-chronic exposure to 25 mg/kg of Na2S2O5 ingestion created a marked detrimental effect on hearing function of rats. Although World Health Organization (WHO) has established an acceptable daily intake (ADI) level of sulfites as 0.7 mg/kg/body weight (24), the daily intake of sulfite may not be in agreement with this value in many cases. Studies have shown that it is possible to consume 180-200 mg/body weight from foods and beverages in a single day [25]. In particular, there are several amino acid preparations utilized in total parenteral nutrition (TPN) solutions that contain large amounts of sulfites [26]. It has been reported that up to 950 mg of bisulfites per day could be administered via TPN solutions [27, 28]. Sulfite toxicity is also considered possible with peritoneal dialysis fluids, some of which contain Na2S2O5 in concentrations of 0.005-0.012% [28]. The generic form of propofol, a drug used by anesthesiologists, contains 25 mg/mL Na2S2O5. Infusion of propofol at a rate of only 50 µg/kg/min is reported to result in toxic levels of sulfite intake within 24 h [29]. The other important point of view is that in considering animal models representing human exposure to sulfites, one must keep in mind that rat liver contains 10-20 fold greater SOX activity than humans [30]. Therefore, median lethal dose (LD50) for acute oral toxicity of Na2S2O5 is reported to be an extremely high level of 1131 and 1903 mg/kg for female and male rats, respectively [24]. In regard, some previous studies used the dose of 25 mg/kg/day and demonstrated this level as a reliable dose for inducing neurotoxicity in rats [11, 12]. For these reasons a higher dose of sulfite (25 mg/kg) than the ADI level was selected in our study to represent human exposure to relatively high levels of sulfites.

Ingestion of 25 mg/kg sulfite for 8 weeks resulted in an apparent deterioration in ABR thresholds and prolongation of all wave latencies (except for I. wave
Auditory brainstem response disturbances in rats induced by sodium metabisulfite ingestion

latency) as well as IPL. I wave latency gives information about the function of the proximal auditory tract; therefore, we can suggest that sulfites have a relatively greater toxic effect on the more central parts of the auditory tract. It is not obvious why this propensity occurs. However, previous studies showed that SOX activity measured in whole brain of some laboratory animals was consistently low compared with other tissues and even in some parts of the brain\(^{14,31}\). Measurement of the expression of SOX in human tissues concurred with this observation. It is demonstrated that a low level of expression pattern was found in all brain regions, cerebellum, cerebral cortex, medulla, spinal cord, occipital pole, frontal lobe, temporal lobe, and putamen, of which the cerebral cortex shows the highest level of expression\(^{35}\). Although there is no information in the literature about SOX enzyme activity in the auditory tract, there may be some differences between locations throughout the pathway. No doubt, this hypothesis needs to be tested by further studies.

There is little information on the mechanism by which accumulation of sulfite affects cellular function. However, free radicals were implicated as increased toxicity of sulfite as one electron oxidation of sulfite would produce a sulfite radical (SO\(_3^-\)), capable of damaging DNA, lipids, and proteins\(^{32}\). This observation further supported by the studies of our group which demonstrate that some antioxidants such as vitamin E can at least in part prevent the oxidative damage of sulfites in various tissues\(^{11,17,33}\). In our experimental study we demonstrated that vitamin E administration together with sulfite ingestion somewhat reduced the changes in ABR values. These results also suggest a sulfite radical mediated toxicity in the auditory neural pathways concurring with the previous studies which implicate the role of free radicals in the toxic mechanisms of sulfites. Indeed, more studies are needed in order to reveal the exact biochemical mechanisms in the auditory pathways.

CONCLUSION

In summary, the presented data confirm that subchronic ingestion of Na\(_2\)S\(_2\)O\(_5\) can cause a marked damage on hearing function of rats. This study, as well as many others, reveals the need for safer preservatives to use in food and chemical industry.

Acknowledgement

This study was supported by a grant from Akdeniz University Research Fund 2001.01.0103.011.

REFERENCES

8. Rencuzogullari E, Ila HB, Kayraldz A, Topaktas, M. Chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes treated
with sodium metabisulfite, a food preservative. Mutat Res 2001;20:107-12.


Auditory brainstem response disturbances in rats induced by sodium metabisulfite ingestion