Study of neurological behaviors resulting from neurotoxicity induced by methyl bromide in rats

Zahraa Mohammed Jasim
Department of physiology, biochemistry and pharmacology, college of veterinary medicine, university of Baghdad
Corresponding author email: zahraa.mohammed1206h@covm.uobaghdad.edu.iq

Rana Abdulla Salih
Department of physiology, biochemistry and pharmacology, college of veterinary medicine, university of Baghdad
Email: Rana.a@covm.uobaghdad.edu.iq

Abstract---Bromide and chloride are chemically related halide elements that the kidney works with to keep the overall body halide content balanced. Also Bromide is a element that known to cause many neurotoxic and other organs toxicity such as GIT tract, respiratory tract and liver. Therefore, we aimed in the present study on the effect of bromide at daily intake doses to investigate possible neurotoxic effect and modulate its possible mechanism and Ameliorative trail of Sodium chloride .Chloride-containing diuretics, In conclusion, the results of the present study indicated that the levels of methyl bromide might induced neurobehavioral changes and disturbance in the oxidant and antioxidant mechanism due to the ability of methyl bromide to accumulate in the brain tissue that causes structural brain damage or disturbing of CNS activity and ameliorative trail of sodium chloride to neurotoxicity

Keywords---neurotoxicity, methyl bromide, brain tissue, rat, Iraq

Introduction

Methyl bromide is an extremely hazardous gas with little olfactory warning (Werner and Nies, 2018). It's a common insecticidal fumigant for dry foods, but it’s poisonous to the central and peripheral nervous systems (Chaudhari et al., 2021). Inhalation causes the majority of methyl bromide intoxication's neurological symptoms. Severe poisoning can cause acute toxicity, which includes headaches, dizziness, stomach pain, nausea, vomiting, and visual problems, as
well as tremor, convulsions, coma, and irreversible brain damage. Neuropathy, pyramidal and cerebellar dysfunction, as well as neuropsychiatric abnormalities, can all result from long-term exposure. Methyl Bromide (MBr) is a gaseous halogenated aliphatic hydrocarbon that is often employed as an insecticide. It is commonly used in the production of dry foods and as a soil fumigant in green houses and fields for nematode, fungus, and weed control (Gamlil, 2020). Humans are exposed to MBr through inhalation and cutaneous contact. Inhalation exposure causes different signs and symptoms depending on the dose and duration of exposure. The neurological system, respiratory system, kidney, eye, and skin are all poisoning targets. Acute MBr poisoning primarily affects the central nervous system (CNS) and is frequently associated with negative outcomes including coma and seizures. Acute intoxication syndrome is combined with visual and hearing impairments, axonal polyneuropathy, ataxia, and psychiatric symptoms in chronic intoxication. Bromide's method of action is extracellular fluid buildup, which causes a toxic syndrome characterized by central nervous system dysfunction, sleepiness, confusion, aggressiveness, erratic behavior, hallucination, and unconsciousness. In central nervous system called bromism (Upadhayay et al., 2020). Methyl bromide has many health effects including the respiratory, neurological, gastrointestinal, dermal, cancer, and developmental effects. In vitro experiments on bacteria, animals, and human cell cultures have demonstrated that methyl bromide has Genotoxic and carcinogenic effects (Teixeira et al., 2022). The importance of neurobehavioral studies in risk evaluation stems from the fact that behavior can be thought of as the net output of the nervous system’s sensory, motor, and cognitive functions, and can serve as potentially sensitive end points of chemically induced neurotoxicity (Kulig et al., 1996).

Used sodium chloride as ameliorative trail for neurotoxicity induced by methyl bromide, however sodium chloride known is an ionic compound with the chemical formula NaCl, and it has a variety of physiological functions in human and animal systems, including regulating body water and osmotic pressure, maintaining acid–base balance, maintaining appropriate blood pressure, and increasing nerve muscle excitability (Ahmed, 2016; Gharban and Al-Shaeli, 2021). Bromide and chloride are chemically related halogens elements that the kidney uses to ensure a normal halide balance in the body. The study’s concept was that the extracellular fluid of the kidney should have a constant halide concentration ratio (iodine, chloride, and bromide). Chloride and bromide anion must be handled in the same way by the kidneys. An increase in chloride consumption causes chloride diuresis (Suryasa, 2021). Bromide excretion in the extracellular fluid will be about similar to chloride excretion. Increased bromide requires a rise in chloride, which is obtained by injecting sodium chloride or ammonium chloride, resulting in chloride diuresis. The times when the chloride was excreted occurred with the times when the bromide was excreted in the same proportion. Our bodies require sodium chloride (NaCl), sometimes known as salt, to function properly (absorb and transport nutrients, control blood pressure, maintain fluid balance, send nerve messages, contract and relax muscles) (Al-hudi, 2011: Ahmed, 2017). Salt is an inorganic chemical, so it is not produced by living organisms. When sodium (Na) and chloride (Cl) mix, white, crystalline cubes are formed. Salt is necessary for body to operate, but too little or too much may be damaging to health (Darwish et al., 2022).
Material and Method

Experimental animals and management

Male albino rats (n=40), over 3-months old and weighing 200-260 g, were procured from the College of Veterinary Medicine / University of Baghdad’s animal house and used in the present study to undertake several tests. Rats were fed a standard pellet diet and given tap water before being placed in special cages in the animal house of the University of Baghdad’s College of Veterinary Medicine for two weeks of adaptation. They were kept in standard conditions of a 12/12 light-dark cycle, (20-25 °C) in an air-conditioned room. The mulch in the bed was changed twice a week. The experiments were carried out in accordance with management ethics. The University of Baghdad submitted laboratory animals for testing, which were conducted under the supervision of a sided committee in the College of Veterinary Medicine.

Methyl bromide and Sodium chloride dosage and dose Preparation

Concentrations of methyl bromide (CH3Br) were generated by dissolving (0.0168 mg) of methyl bromide (CH3Br) in 60 ml distilled water and administering at a dosage volume of 0.0028 ml/100gm.BW to the (B,C,D) animals group. While preparing the NaCl concentration of 5 g/ml, 30 ml distilled water was added to the (C) concentration (15 g/ml) and administered at dose volumes of 0.1 ml/100gm.BW to the (C) animal group and 1.8 ml/100gm.BW to the (D) animal group. The control group (A) received 0.1 ml D.W/100 g BW by oral route daily for 28 days using a stainless steel garage needle.

Experimental Design

Male albino rats (n=40) were separated into four groups (n=10), one control group, one toxic group, and two treatment groups (low dose and high dosage), each of which was assigned to one of the following dosing regimens: (A) Control: Ten rats were given 0.1 ml/100 g D.W/ orally every day with a stainless gavage needle. (B) Ten rats were given a daily dose of methyl bromide of 1 ml/100 g B.W., which corresponded to the acceptable daily intake (ADI) of MB. (C) Ten rats were given MB at a dose of 1 ml/100 g B.W. daily and Nacl at a dose of 0.6 ml/100 g B.W. daily orally. (D) Ten rats were given MB at a dose of 1 ml/100 g/B.W/ daily and Nacl at a dose of 1.8 ml/100 g/B.W/ daily orally. During this investigation, numerous parameters were measured and dosing continued for four weeks

Neurobehavioral test

Were performed including (head poking, rotaroad test, swimming ranks, negative geotaxis, cliff avoidance, Y-Maze tests) at 2 weeks and 4 weeks for all groups.

Negative geotaxis test

In this test, a slanted-wooden piece with a 45-degree angle was utilized, and the rat was placed with its head down on the surface figure(3.1), then the time it took
for the rat to make a 180-degree rotation was recorded; the maximum time allowed was 60 seconds (Hasan, 2021).

**Cliff avoidance test**

This test was carried out by placing a rat on a table with a 9 cm border and recording the time it took for the rat to turn away (Mohammed, 1984).

**Swimming rank test**

This test was carried out in a glass pool with a depth of 30 cm and warmed water at 30°C, with each rat swimming for 10 seconds and the grades of swimming being evaluated as shown in (figure 3-3). (Vohress et al., 1979).

- Grade 0: the nose is under the plane of water.
- Grade 1: the nose with plane or above water.
- Grade 2: the nose and crown with or above the plane of water while the ear is under.
- Grade 3: as in grade (2) but the plane of water at the mid of ears.
- Grade 4: as in grade (3) but the plane of water under the ears

**Y- Maze test**

This test was carried out using a Y-shaped wooden contraption (Whishaw and Tomie, 1997). In addition, the Y maze was created according to the instructions provided by (Dellu et al., 1992). The arm-recognition component of the Y maze has been employed since the hippocampus is not required for object-recognition memory (Pinel et al., 1992). The Y maze has three identical arms, each measuring 50 cm in length, 16 cm in width, and 32 cm in height. Two pairs of infrared photocells were installed in each arm, 21 cm and 42 cm from the arms' ends and 4.25 cm above the floor. The apparatus arms were numbered A, B, and C; the A arm was used to start the test; the rat was placed in the A arm and the 12 points were measured. Successful trail is the entry to the 3 distinct arms animal admittance to other arms, and choose the three different trials (Rawlins and Deacon, 2006).

**Head pocking design**

It was carried out by carving nineteen punchers in wood (diameter 100 cm for adult rats) and recording the rate of head entries into pores over a three-minute period (Al-Bakkoh, 2002)

**Rota Rod test**

**Rota Rod design**

The scholar modified the Kymograph by adding a 10 cm diameter cylinder for adult rats and covering it with rough ringed paper; the speed of the instrument was 29 revolutions per minute in adult rats. The time it takes an animal to attain coordination on the Rota rod apparatus without feeling down is called the Rota Rod test time (Al-Bakkoh, 2002).
**Result and Discussion**

**Negative geotaxis**

The result of negative geotaxis appeared significant increase (P ≤ 0.05) in all groups throughout the period of study after dosing (2 weeks and 4 weeks) in comparison with control group, between groups in 2 weeks Significant increase (P ≤ 0.05) compared G1, G2 and G4 , Significant decrease (P ≤ 0.05) at G1 and G4 . There is significant increase (P ≤ 0.05) in G4 compared G1.

Table (4-2): Negative Geotaxis Test (up position / 60 seconds) of methyl bromide dose and different dosed sodium chloride rat groups and control on at different experimental periods

<table>
<thead>
<tr>
<th>Groups</th>
<th>Periods (mean± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
</tr>
<tr>
<td>G1 Control</td>
<td>2.08 ± 0.19 Ad</td>
</tr>
<tr>
<td>G2 MBr (0.028 mg/kg)</td>
<td>14.80 ± 1.51 Ab</td>
</tr>
<tr>
<td>G3 MBr (0.028 mg/kg)+Nacl (1 g/kg)</td>
<td>18.90 ± 2.32 Aa</td>
</tr>
<tr>
<td>G4 MBr (0.028 mg/kg)+Nacl (3 g/kg)</td>
<td>6.30 ± 0.66 Ac</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
</tr>
</tbody>
</table>

Means with a different small letter in the same column are significantly different (P≤0.05).
Means with a different capital letter in the same row are significantly different (P ≤ 0.05).

![Figure (4-1): Negative geotaxis test](image-url)
Cliff avoidance test

The result of cliff avoidance that listed in table (4-3) appeared no significant increase (p ≤ 0.05) between 4 weeks as compared 2 weeks whereas no significant decrease (P ≤ 0.05) in G3 and G4 compared with 2 weeks, between groups there is significant increase (P ≤ 0.05) in G2 compared with G1, G3, G4 in 2 weeks and 4 weeks while there is no significant increase (P ≤ 0.05) in G3 and G4 compared control in 2 weeks and 4 weeks.

Table (4-3): Cliff avoidance test (avoid falling/20sec) of methyl bromide dose and different dosed sodium chloride rat groups and control on at different experimental periods

<table>
<thead>
<tr>
<th>Groups</th>
<th>periods (mean± SE)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
<td>4 weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.50 ± 0.22 Ab</td>
<td>3.60 ± 0.28 Ab</td>
<td></td>
</tr>
<tr>
<td>G1 Control</td>
<td>47.10 ± 0.39 Aa</td>
<td>50.90 ± 8.35 Aa</td>
<td></td>
</tr>
<tr>
<td>G2 MBr (0.028 mg/kg)</td>
<td>8.70 ± 1.05 Ab</td>
<td>7.80 ± 0.96 Ab</td>
<td></td>
</tr>
<tr>
<td>G3 MBr (0.028 mg/kg)+Nacl (1g/kg)</td>
<td>8.20 ± 0.86 Ab</td>
<td>6.80 ± 0.69 Ab</td>
<td></td>
</tr>
<tr>
<td>G4 MBr (0.028 mg/kg)+Nacl (3g/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>11.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means with a different small letter in the same column are significantly different (P≤0.05)
Means with a different capital letter in the same row are significantly different (P≤0.05)

Figure (4-2): Cliff avoidance test
**Y-Maze test**

The results of y-maze in listed in table (4-4) that appeared significant decrease (p<0.05) within G2 in 2 weeks and 4 weeks, no significant decrease (p<0.05) in G3 and G4 at 2 weeks and 4 weeks between groups significant decrease (p<0.05) in G2 at 4 weeks compared with G1

Table (4-4): Y-Maze test (3min) of methyl bromide dose and different dosed sodium chloride rat groups and control on at different experimental periods

<table>
<thead>
<tr>
<th>Groups</th>
<th>periods (mean± SE)</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Control /D.W</td>
<td></td>
<td>5.1 ± 0.14 Aa</td>
<td>4.9 ± 0.12 Aa</td>
</tr>
<tr>
<td>G2 MBr (0.028 mg/kg)</td>
<td></td>
<td>4.5 ± 0.09 Aa</td>
<td>3.4 ± 0.07 Bc</td>
</tr>
<tr>
<td>G3 MBr (0.028 mg/kg)+Nacl (1 g/kg)</td>
<td></td>
<td>3.9 ± 0.09 Ac</td>
<td>3.8 ± 0.10 Ac</td>
</tr>
<tr>
<td>G4 MBr (0.028 mg/kg)+Nacl (3 g/kg)</td>
<td></td>
<td>3.7 ± 0.10 Ac</td>
<td>3.5 ± 0.07 Ac</td>
</tr>
<tr>
<td>LLSD l LSD</td>
<td></td>
<td></td>
<td>1.063</td>
</tr>
</tbody>
</table>

Means with a different small letter in the same column are significantly different (P≤0.05)
Means with a different capital letter in the same row are significantly different (P≤0.05)

**Rotaroad test**

The result of rotaroad that listed in table (4-5) appeared no significant increase (p≥0.05) in G1 at 4 weeks compared 2 weeks, significant decrease (p≤0.05) in G2
at 4 weeks compared 2 weeks, significant increase (p≤0.05) in G3 and G4 at 4 weeks compared 2 weeks. Between groups there is significant decrease (p≤0.05) in G2, G3, G4 compared with G1 at 2 weeks and 4 weeks.

Table (4-5): Rotaroad test (Run/3 min) of methy bromide dose and different dosed sodium chloride rat groups and control on at different experimental periods

<table>
<thead>
<tr>
<th>Groups</th>
<th>periods (mean± SE)</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1  Control</td>
<td></td>
<td>122.00 ± 7.27 Aa</td>
<td>131.20 ± 7.37 Aa</td>
</tr>
<tr>
<td>MBr (0.028 mg/kg)</td>
<td></td>
<td>65.30 ± 3.45 Ac</td>
<td>31.50 ± 2.11 Bd</td>
</tr>
<tr>
<td>MBr (0.028 mg/kg)+Nacl (1 g/kg)</td>
<td></td>
<td>60.30 ± 2.86 Bc</td>
<td>87.60 ± 4.60 Ac</td>
</tr>
<tr>
<td>MBr (0.028 mg/kg)+Nacl (3 g/kg)</td>
<td></td>
<td>92.40 ± 4.18 Bb</td>
<td>118.00 ± 6.14 Ab</td>
</tr>
</tbody>
</table>

LSD 14.38

Means with a different small letter in the same column are significantly different (P≤0.05)
Means with a different capital letter in the same row are significantly different (P≤0.05)

**Swimming rank test (Grade/10 seconds)**

The result of swimming rank test that listed in table (4-6) appeared significant decrease (p≤0.05) in G2 at 4 weeks compared 2 weeks, no significant increase (p≤0.05) in G3 and G4 at 4 weeks compared 2 weeks between groups there is no significant increase (p≤0.05) in G3 and G4 compared G2 at 2 weeks, significant increase (p≤0.05) in G3 and G4 compared G2 at 4 weeks.
Table (4-6): swimming rank test (Grade/ 10 seconds) of methyl bromide dose and different dosed sodium chloride rat groups and control on at different experimental periods

<table>
<thead>
<tr>
<th>Groups</th>
<th>periods (mean± SE)</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Control /D.W</td>
<td>3.90 ± 0.10 Aa</td>
<td>3.80 ± 0.13 Aa</td>
</tr>
<tr>
<td>G2</td>
<td>MBr (0.028 mg/kg)</td>
<td>2.44 ± 0.24 Ab</td>
<td>1.67 ± 0.37 Bb</td>
</tr>
<tr>
<td>G3</td>
<td>MBr (0.028 mg/kg)+Nacl (1 g/kg)</td>
<td>2.80 ± 0.20 Ab</td>
<td>3.20 ± 0.20 Aa</td>
</tr>
<tr>
<td>G4</td>
<td>MBr (0.028 mg/kg)+Nacl (3 g/kg)</td>
<td>3.00 ± 0.21 Ab</td>
<td>3.40 ± 0.27 Aa</td>
</tr>
</tbody>
</table>

Means with a different small letter in the same column are significantly different (P≤0.05)
Means with a different capital letter in the same row are significantly different (P≤0.05)

Figure (4-5): swimming rank test

Head packing test (3 minute)

The result of head packing test that listed in table (4-7) appeared no significant increase (p≤0.05) in G3 and G4 at 4 weeks compared to 2 weeks, no significant decrease (p≤0.05) in G2 at 4 weeks compared at 2 weeks. Whereas between groups there is significant decrease (p≤0.05) in G2 and G3 compared with G1 at 2 weeks and 4 weeks.

Table (4-7): Head packing test (3 minute) of methyl bromide dose and different dosed sodium chloride rat groups and control on at different experimental periods
From the examined neurobehavioral tests, it was clear that there was effect of MBr in groups in the periods of study which indicating the presence of clear defect either on the structure of the nervous system or on the neurotransmitters both centrally and peripherally. This effect was reflected the changes in locomotor activity. We must not exclude the effect of other neurotransmitters that affects the mood and memory, locomotion, vestibular, function and emotion, like dopamine, epinephrine and norepinephrine, histamine and serotonin. Both centrally and peripherally that might also affect by methyl bromide intake in human or animal. Bromide's effects may extend beyond the lungs and into the cardiovascular system, according to research. We postulated in this work that Br exposure promotes the creation of brominated moieties, which may circulate to the brain via the bloodstream and cause brain injury by oxidative effects and regulation of neurotransmitter and precursor levels. Abnormalities in their catecholamine metabolism in the brain might potentially be the cause of their behavioral changes. Catecholamines are involved in a variety of physiological processes, including cardiovascular, endocrine, pulmonary, renal, and neurotransmission. Catecholamines are hormones that are created endogenously in neurons and
released in axons, dendrites, and peripheral organs like the adrenal medulla. They have an important function in memory (learning, addiction, neural transmission, autonomic functioning, and behavioral control) (Nolan and Gaskil, 2019). Dopamine (DA) is a neuroendocrine transmitter belonging to the catecholamine and phenethylamine families. DA in the brain plays a critical role in modulating a variety of actions on neuroendocrine and behavioral systems. Pathological change in DA transmission is a key feature of many neurological and psychiatric disorders, such as schizophrenia, Parkinson’s disease, Tourette’s syndrome, and many others. Theories on the behavioral function of DA in the brain have advanced from the original proposal that DA could mediate the hedonic impact of rewards and motor behavior, more recently it has been proposed that DA mediates reinforcement learning. Even if the theoretical debate around DA function continues, the central role of this neurotransmitter as a mediator of memory formation is being widely recognized (Elvira De Leonibus et al. 2015).

Epinephrine (adrenaline) and nor-epinephrine (nor-adrenaline) disturbance have all been linked to brain damage (Chen et al., 2017). Alterations Bromide has been linked to lung damage and altered lung function (Matalon et al., 2016). We looked at neurotransmitters after Br exposure by looking at catecholamine levels in the brainstem, which houses the respiratory center (Martinez et al., 2016). One of the necessary amino acids involved in the synthesis of serotonin is tryptophan, and tryptophan hydroxylase, or TrpH, is a rate-limiting enzyme required for this conversion. Serotonin produces 5-hydroxyindoleacetic acid (5-HIAA), which is a byproduct. Tyrosine, on the other hand, is a non-essential amino acid that serves as a precursor to important neurotransmitters such as L-DOPA, dopamine, adrenaline, and norepinephrine. TyrH is an enzyme responsible in the conversion of tyrosine to L-DOPA, which is then converted to dopamine, a significant neurotransmitter. Homovanillic acid (HVA) is a downstream metabolite of dopamine. All of these neurotransmitters have a role in neural transmission as well as mood regulation. To assess these compounds, brainstems from unexposed and Br2-exposed rats were processed for HPLC (high-performance liquid chromatography). The findings revealed an increase in serotonin (5-HT), one of the most important chemicals involved in mood swings (Wilson et al., 2021). The result in table (4-4) of Y-maze test because Because methyl bromide may rapidly enter the CNS and impact the physiological function of neurotransmitters like GABA, this is the effect. As a result, it has the ability to affect both neurological and behavioral processes. The GABAA receptor, which is a chloride channel, has been shown to be stimulated by Br. It causes hyperpolarization of neurons and an inhibitory impact in the brain when activated. In this study, memory impairment was linked to KBr-induced GABA receptor activation. According to a large number of studies, an increase in GABA concentration or receptor activation causes memory impairment. (Rabbani and Sharifabad, 2017). While the result in table (4-5) of rotaroad test it This result indicates dysfunction of the peripheral nerves system (KISHI et al., 1980). However the result in table (4-6) of swimming rank test appeared clearly that methyl bromide caused damages in central and peripheral nervous system that change neurotransmitters level accordingly with methyl bromide dose in rats. Whereas the result in table (4-7) of head packing test appeared MBr Alteration in serotonergic transmission may also contribute in bromide-induced cognitive impairment, according to this finding. In vitro investigations have shown that bromide decreases serotonin release in this way. Furthermore, serotonin interacts
with cholinergic, glutamatergic, dopaminergic, and GABAergic systems to play a role in a wide range of activities, including learning and memory. As a result, bromide suppression of serotonergic activity can lead to changes in other neurotransmitters, which might lead to memory problems (Rabbani and Sharifabad, 2017).

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