

## Biochemical effects of induced neurotoxicity by methyl bromide in rats

<sup>1</sup>Zahraa Mohammed. Jasim , <sup>2</sup>Rana Abdulla Salih

Department of physiology, biochemistry and pharmacology, college of veterinary medicine, university of Baghdad

<sup>1</sup>Email: [zahraa.mohammed1206h@covm.uobaghdad.edu.iq](mailto:zahraa.mohammed1206h@covm.uobaghdad.edu.iq)

<sup>2</sup>Email: [Rana.a@covm.uobaghdad.edu.iq](mailto: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 by measuring the oxidant and antioxidant impact in brain and Ameliorative trail of Sodium chloride .Chloride-containing diuretics, such as sodium chloride, increase bromide elimination in the urine.

**Key word: neurotoxicity , methyl bromide , Rat .**

### I. INTRODUCTION

The negative effect of a biological, chemical, and physical agent on the central or peripheral nerve systems' structure or function is called neurotoxicity and it is a disorder that disturbance of normal function in such a way that permanent or reversible damage to neural tissue of the nervous system(Catarina *et al.*, 2008). Methyl bromide is a gaseous halogenated aliphatic hydrocarbon that is often employed as an insecticide. Methyl bromide is commonly used in the production of dry foods and as a soil fumigant in greenhouses and fields for nematode, fungus, and weed control (Gamliel, 2020). Methyl bromide is an extremely hazardous gas with little olfactory warning (Werner and Nies, 2018). The first reported case of MeBr poisoning was in 1893 (Bulathsinghala and Shaw, 2014). Exposure route of methyl bromide is inhalation and dermal so that Inhalation causes the majority of methyl bromide intoxication's neurological symptoms. Acute MBr poisoning primarily affects the central nervous system (CNS) and is frequently associated with negative outcomes including coma and seizures, headaches, dizziness, stomach pain, nausea, vomiting, and visual problems, as well as tremor, convulsions and irreversible brain damage. Neuropathy, pyramidal and cerebellar dysfunction, as well as neuropsychiatric abnormalities, can all result from long-term exposure (Suwanlaong and Phanthumchinda, 2008). 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** (Joshi *et al.*, 2020). Methyl bromide have many health effects are 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 (Edwards and Crambert, 2017). 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. 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). 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)

## II. MATERIALAND METHODS

### 1-Antioxidant parameter MDA

The concentration of MDA in serum was determined according Baege and aust methods (nur *et al.* , 2013).

A. MDA formed from breakdown of polyunsaturated fatty acids serves as a convenient index of peroxidation reaction. The thiobarbituric acid method was used to estimate the MDA, which reacts with thiobarbituric acid (TBA) giving pink color read at  $\lambda$  max 535 nm (Nasif, 2002).

### B. Reagents



Thiobarbituric acid 0.188 gm. of (0.013 N) and 7.5 gm. tri-chloro acetic acid (TCA) (0.46 N) was dissolved in a suitable volume of hydrochloric acid (HCl) (0.25 N), the mixture was shaken and heated at 70 °C until dissolved completely then the volume was made up 100 ml with HCL (0.25N).

### C. Procedure

1. One ml of the reagent was added to 0.5 ml of serum sample.
2. The tube was mixed well by vortex, and then heated at 70 °C for 20 min.
3. After cooling, the mixture was centrifuge for 10 min at (6000 rpm) by using microfuge, centrifuge.
4. The clear supernatant was read at  $\lambda$  max 535 nm against the blank, which contains 1ml of D.W and 0.5 ml of reagent.

### D. Calculation of Results

$$\text{Serum MDA concentration } (\mu\text{mol/l}) = \frac{\text{Abs} \times \text{D.F}}{\epsilon \times \text{d}}$$

Abs: Absorbance

d: Light path = 1 cm

D. f: Dilution factor = 3

$\epsilon$ : Extinction coefficient =  $1.56 \times 10^5 \text{M}^{-1} \text{cm}^{-1}$

NO	Companies	Chemicals
1-	BDH chemical Ltd., England	
		Thio barbituric acid (TBA) (C <sub>3</sub> H <sub>3</sub> SN <sub>2</sub> )
		TriChloro Acetic acid (TCA) (C <sub>2</sub> HO <sub>2</sub> Cl <sub>3</sub> )

### 2- antioxidant parameter GSH.

The serum thiol concentration was measured according to the ellman method(Nasif ,2002).

#### A- Reagents:

(a) H<sub>2</sub>NaPO<sub>4</sub> (0.2M) was prepared by dissolving (0.2gm) in (100ml) D.W.

(b)HNa<sub>2</sub>PO<sub>4</sub> (0.2M) was prepared by dissolving (0.2gm) in (100ml) D.W.

1) **Reagent A:** [Phosphate buffer (0.2M); pH= 7] was prepared by mixing 41 ml of (b) with 9 ml of (a). Volume was completed to 100ml by D.W. and pH was adjusted.



2) **Reagent B:** [Phosphate buffer (0.2M); pH= 8] was prepared by mixing 5 ml of (a) with 45 ml of (b) and completed to 100ml by D.W. pH was adjusted before and after add D.W.

3) **Reagent C (DTNB reagent):**This solution prepared by dissolving 39.6 mg of DTNB in 10ml of reagent A with added tiny amount of Na<sub>2</sub>CO<sub>3</sub>.

#### **B- Procedure:**

1) 20 µl of serum was added to 1000 µl of D.W in a test tube.

2) Then 1000 µl of reagent B was added and mixed well.

3) 1500 µl was draw from above mixture and 200 µl of reagent C was add. The solution mix well and incubated at 37°C for 60 min.

4) Blank was prepared as the same steps in (1, 2 and 3) with the exception that the same volume of D.W. was added instead of serum in step 1.

5) The absorbance was read at λ= 420nm.

#### **C- Calculations:**

$$GSH \text{ con. in serum } \mu\text{mol/L} = (T - B) \times \frac{d.f}{\epsilon} \times 10^6$$

T: Test absorbance

ε: Extinction coefficient = 13600 M<sup>-1</sup>.cm<sup>-1</sup>

B: Blank absorbance

d.f: Dilution factor =102.

#### **3-Serum level of sodium and chloride**

The levels of Na<sup>+</sup> and Cl<sup>-</sup> in serum samples were examined Using the Ion-Selective Electrode (ISE) technique, the AVL 9180 Electrolyte Analyzer .blood sample was inserted into the vacutainer tube of the clot separator gel during the procedure. At room temperature, the blood was allowed to coagulate. The sample serum is then divided into two groups. The first sample group was examined immediately, whereas the second sample group was kept in the refrigerator. The serum was kept in the fridge for 2 and 3 hours at 4°C 2°C. Before the test, the serum was allowed to sit at room temperature for 30 minutes (Trisna *et al.*, 2018). The samples were centrifuged for 10 minutes at 3,000 rpm. Eppendorf tubes were used to separate the serum. The samples used were neither hemolytic, icteric, or lipemic, and were visually clear.

### **III.RESULT AND DISCUSSION**

#### **Serum level Chloride and sodium.**

The result in list table (1-1) :showed significance increase in G2 of chloride level compared with control group and significance decrease in G3 and G4 compared with G2, significance decrease P(≤0.05) in G3 and G4 compared with G2 . whereas showed significance increase P(≤0.05) in G3 and G4 of sodium level compared with G2 and G1.



Table (1-1): serum chloride and sodium level

Parameters Groups	Serum level parameters (mean±SE)	
	sodium (meq/L)	chloride(meq/L)
Control/D. W	138.50 ±0.96 c	84.78±1.06 b
G2 MBr(0.028mg/kg)	139.87 ±1.10 c	99.00±0.88 a
G3 MBr(0.028mg/kg)+Nacl(1g/kg)	153.98 ±0.54 b	75.27±0.88 c
G4 MBr(0.028mg/kg)+Nacl(3g/kg)	170.36 ±1.31 a	68.28±1.06 d
LSD	3.091	2.96

Means with a different small letter in the same column are significantly different ( $P \leq 0.05$ )

Means with a different capital letter in the same row are significantly different ( $P \leq 0.05$ )

This result showed in G2 increased serum chloride concentration and serum sodium level paralleled to normal. This indicator bromide toxicity (Fantinat *et al.*, 2021). Significance decrease in G3 and G4 compared with G2 due to Dietary chloride may improve competition for tubular reabsorption between chloride and bromide and accelerate bromide excretion by causing a significant drop in G3 and G4 relative to G2. In various tissues, sodium-chloride co-transport and anion exchange pathways differ in terms of susceptibility to blocking drugs and affinity for anions (Warnock *et al.*, 1984). Anion exchangers, on the other hand, have a low anion selectivity and may transport a variety of anions (bromide, iodide, phosphate, and sulphate) (Stein, 1986). Fluid reabsorption is anion dependent. The capacity of diverse anions to facilitate fluid reabsorption is more consistent with an anion exchanger than with a sodium-chloride co-transport mechanism for salt reabsorption. Anion transport through the proximal tubular epithelium can occur between cells or across cells (transcellular) (paracellular). The removal of driving gradients would inhibit anion movement if movement is predominantly paracellular. Transcellular anion movement must take place across two distinct cell membranes, each of which may contain a variety of transport mechanisms capable of swapping one anion for another or moving anions and cations in tandem (Giebisch and Aronson, 1986). Due to sodium load daily intake, the findings indicated a significant rise in G3 and G4 serum sodium levels when compared to the control (Grillo *et al.*, 2019).



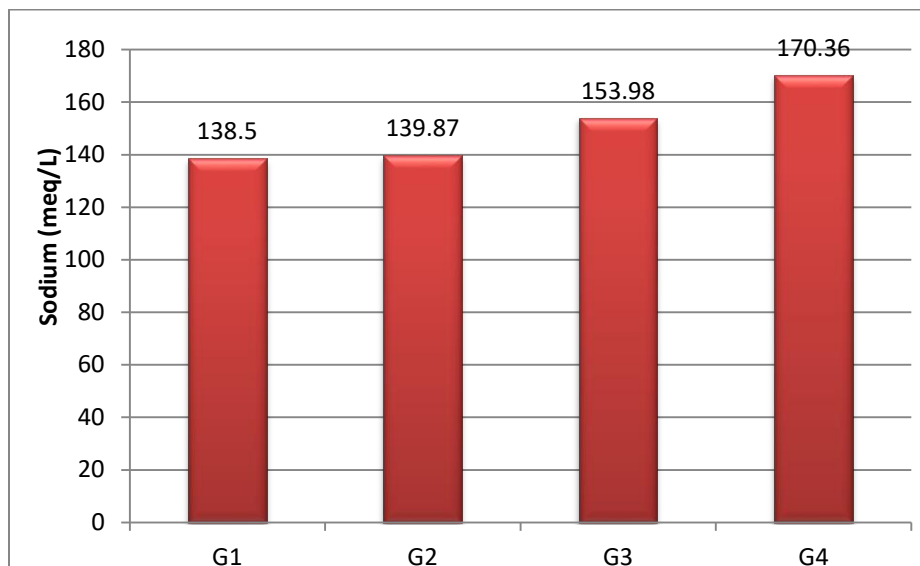


Figure (1-1): serum sodium level

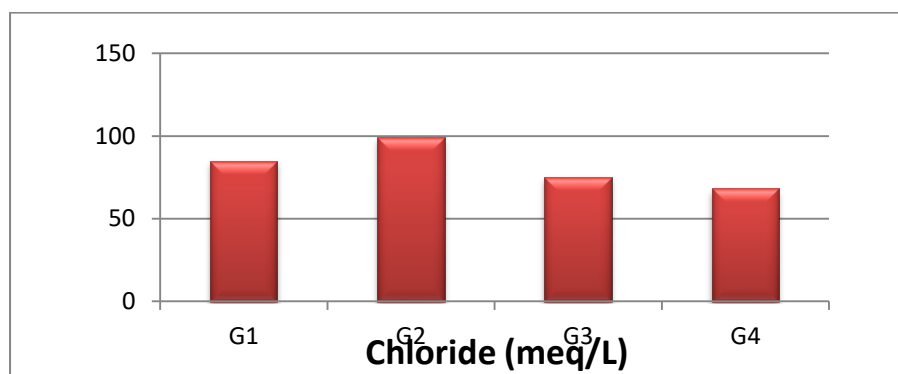


Figure (1-2): serum chloride level

#### Anti-Oxidant parameter.

The results in list table (1-2): showed significance increase in MDA in all groups compared with G1 Control group and significance decrease in GSH in all groups compared with G1 Control group.

Table (1-2): Oxidative stress parameters

Parameters Groups	Oxidative stress parameters (mean±SE)	
	MDA (µmol/L)	GSH(µmol)
G1 Control/D. W	2.15 ±0.05 <b>d</b>	420.00±21.17 <b>a</b>
G2 MBr(0.028mg/kg)	7.50 ±0.38 <b>a</b>	220.00±6.79 <b>c</b>
G3 MBr(0.028mg/kg)+Nacl(1g/kg)	5.00 ±0.30 <b>b</b>	380.00±10.70 <b>b</b>
G4 MBr(0.028mg/kg)+Nacl(3g/kg)	3.88 ±0.36 <b>c</b>	412.85±8.18 <b>a</b>
LSD	0.988	33.74

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)

Demonstrated that Br exposure altered antioxidant enzyme activities and antioxidant levels, indicating that MDA were elevated as defensive mechanisms. In aerobic organisms, a certain number of reactive oxygen species (ROS) are created as by-products of normal metabolic processes, such as superoxide anions O<sub>2</sub><sup>-</sup>, hydrogen peroxides (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radicals (·OH), and a balance is maintained between ROS generation and clearance (Ken *et al.*, 2003). Organisms can respond to increased ROS generation by up regulating antioxidant defense, and GSH plays a critical role in cellular detoxification metabolism for anti-oxidation and detoxification (Zhang *et al.*, 2015). Lipid peroxidation, a process between free radicals and unsaturated fatty acids in cellular membranes, produces MDA (Morales *et al.*, 2004) Increased MDA level is an important indicator of lipid peroxidation



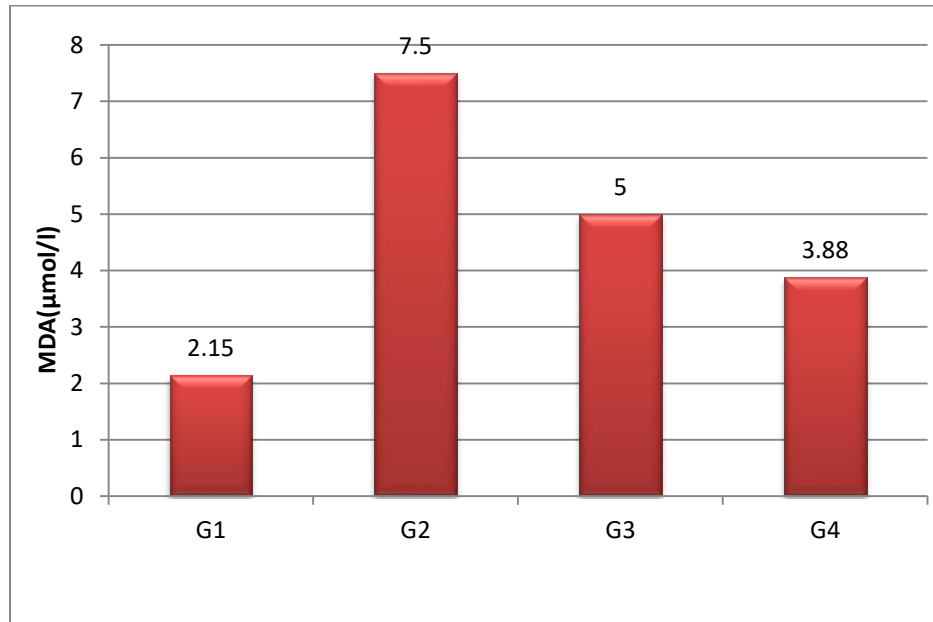


Figure (4-10): Oxidative stress parameters MDA

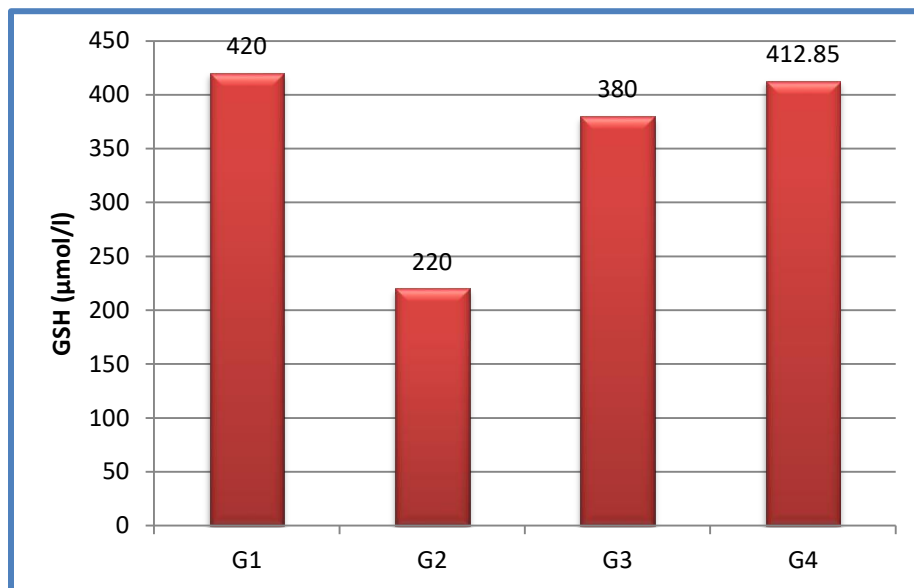


Figure (4-11): Oxidative stress parameters GSH



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