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Pilot and Acute Toxicity Studies on Sodium Chloride Administered Orally in Wistar Rats

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Abstract
Salt, also known as sodium chloride (NaCl), is about 60% chloride and 40% sodium. It flavors and preserves food. It is also used as a binder and stabilizer. The human body requires a minute amount of sodium to maintain the proper balance of water and minerals, conduct nerve impulses, contract and relax muscles. It is estimated that human need about 500 mg of sodium daily for these functions. A pilot and acute toxicity studies were carried out to determine the LD100 and LD50 of sodium chloride administered orally. A total of 34 Wistar rats weighing between 150-160 g were used in this study. Ten (10) rats were used for the determination of LD100 of sodium chloride administered orally with the following doses as 0.0 g/kg, 2.5 g/kg, 5.0 g/kg, 10.0 g/kg, 15.0 g/kg, 20.0 g/kg, 22.5 g/kg, 30.0 g/kg and 40.0 g/kg respectively. In the acute toxicity study, 24 rats were divided into six (6) groups of 4 rats each. They were treated orally with the following doses as 0.0 g/kg, 2.5 g/kg, 5.0 g/kg, 10.0 g/kg, 15.0 g/kg and 20.0 g/kg respectively. From these findings, the LD100 and LD50 obtained were found to be 20.0 g/kg and 13.12 g/kg respectively. Based on the LD50 obtained, sodium chloride could be classified as practically non-toxic substance according to Matsumura toxicity rating of chemical when administered orally.

Keywords: Pilot, Acute, Toxicity, LD50, Ld100

1. Introduction

Salt, also known as sodium chloride (NaCl), is about 60% chloride and 40% sodium. It flavors and preserves food. It is also used as a binder and stabilizer. The human body requires a minute amount of sodium to maintain the proper balance of water and minerals, conduct nerve impulses, contract and relax muscles. It is estimated that human need about 500 mg of sodium daily for these functions (He and MacGregor, 2019; Stallings *et al*., 2019).

NaCl is the oldest spice in human history and has a multifunctional role in the modern-day food industry and biotechnology (Soto-Escageda *et al*., 2016). There has been a considerable increase in the salt content of foods due to changes in human dietary habits vis-à-vis high consumption of industrialized, processed, and fast foods (Ni Mhurchu *et al*., 2011; Korosec and Pravst, 2014; Brouillard *et al*., 2020). However, industrial suitability, gustatory delights, salt addiction, and consumer's acceptability are a few of the factors still influencing the continued demand, interest, and consumption of high salt diet worldwide (Anderson *et al*., 2010; Kloss *et al*., 2015). Sodium is involved in several trans-membrane and physiological processes and is dominantly supplied via dietary salt (Grau *et al*., 2014; Ayed *et al*., 2021). Unfortunately, uncontrolled and excessive consumption of salt has been linked to the development of cardiovascular disorders, endothelial dysfunction, and derangement in lipid metabolism (Wang *et al*., 2015; Han *et al*., 2018 & He *et al*., 2020). Increased activities of reactive oxidative species (ROS), infiltration of immune cells, and glomerular hyper-filtration have been postulated as the likely mechanisms of high salt-induced renal damage and hypertension (Shimosawa, 2013; Mattson, 2014; Fehrenbach *et al*., 2019; He *et al*., 2020).

In toxicity study, the establishment of LD100 and LD50 in order to rate the substance of interest is of Paramount important. Moreover, most of the studies on toxicity of sodium chloride did not take that into consideration. Therefore, the aim of this study is to determine the LD100 and LD50 of sodium chloride for oral route of administration through pilot and acute toxicity studies. The pilot toxicity study is used to establish the minimum dose that cause 100% death (LD100) while the acute toxicity study provides the minimum lethal dose that killed 50% (LD50) of the study animals (Matsumura, 1975).

Material and Methods

Materials

Materials used in this research study include 2 ml and 5ml hypodermic syringes, gauge tube, sodium chloride which was purchased in solute form from our local shops, Nigeria.

Experimental Animals

A total of 34 Wistar rats weighing between 150-160 g were used in this study. They were allowed to acclimatize for two weeks before the commencement of the experiment. They were fed pelletized growers' feed (Vital Feed, Jos Nigeria) and allowed access to water ad liblitum throughout the experiment period. The experimental protocol was approved by the Ethical committee of the Usmanu Danfodiyo University, Sokoto, Nigeria.

Preparation of sodium chloride (NaCl) for pilot and acute toxicity study

For oral administration, 15.0 g of the sodium chloride was dissolved in 40.0 ml of sterile water. This indicates that 1.0 ml of the solution contains 0.375 g of sodium chloride.

Dosages and administration of sodium chloride (NaCl) for pilot study

A total of ten (10) Wistar rats were used for the determination of LD100 of sodium chloride administered orally in the pilot study. The doses were as follows; group 1 received 0.0 g/kg, group 2 received 2.5 g/kg, group 3 received 5.0 g /kg, group 4 received 10.0 g/kg, group 5 received 15.0 g/kg, group 6 received 20.0 g/kg, group 7 received 22.5 g/kg, group 8 received 30.0 g/kg, group 9 received 35.0 g/kg and group 10 received 40 g/kg respectively.

Dosages and administration of sodium chloride for acute toxicity study

A total of twenty-four (24) Wistar rats for acute toxicity study in orally treated rats were divided into six (6) groups of four (4) rats each. The doses were as follows; group 1 received 0.0 g/kg, group 2 received 2.5 g/kg, group 3 received 5.0 g/kg, group 4 received 10.0 g/kg, group 5 received 15.0 g/kg and group 6 received 20.0 g/kg respectively.

Determination of LD50 of sodium chloride

The LD50 of sodium chloride administered orally was obtained using the arithmetic method of Karber after determining the LD100 from the pilot study in orally treated rats. The arithmetic method of Karber for calculating LD50 was as follows.

LD50 = LD100 - (Sum of dose diff. x mean dead/No of rats).

Results

After the administration of the sodium chloride in the pilot study, the treated rats were monitored within 24 hours for signs and symptoms of sodium chloride toxicity such as sedation, respiratory distress, pigmentation, coma before death occurred.

From the pilot study carried out, the minimum dose that caused death (LD100) in the animals was 20.0 g/kg for orally treated rats (Table 3.1).

Table 3.1: Determination of minimum Dose that cause 100% Death (LD100) of Sodium Chloride Orally Treated Rats

Groups	No of Rats	Volume (ml)	Dose (g/kg)	Dead
1	1	0.0	0.0	NO
2	1	1.0	2.5	NO
3	1	2.0	5.0	NO
4	1	4.0	10.0	NO
5	1	6.0	15.0	NO
*6	1	*8.0	*20.0	YES
7	1	9.0	22.5	YES
8	1	11.0	30.0	YES
9	1	13.0	35.0	YES
10	1	15.0	40.0	YES

***M**inimum Dose that cause 100% Death (LD100)

The LD50 for orally treated rats were determined using Karber arithmetic method after LD100 determination. Signs and symptoms of sodium chloride toxicity such as sedation, respiratory distress, pigmentation, coma before death were observed. It was shown that death occurred at group 4, 5 and 6.

By applying the arithmetic method of Karber, the LD100 is 20.0 g/kg, the sum of dose difference x mean of dead = 27.5, number of rats per group = 4. Therefore, LD50 = 20 - (27.5/4) = 20 - 6.88 = 13.12 g/kg (Table 3.2).

Table 3.2: Determination of Median Lethal Dose (LD50) for Sodium Chloride Orally Treated Rats

Groups	Dose (g/kg)	No of Rats	Dose Diff.	No of Dead	Mean of Dead	Dose Diff. X Mean of Dead
1	0.0	4	0.0	0	-	-
2	2.5	4	2.5	0	-	-
3	5.0	4	2.5	0	-	-
4	10.0	4	5.0	1	0.5	2.5
5	15.0	4	5.0	2	1.5	7.5
6	20.0	4	5.0	4	3.5	17.5
						Total = 27.5

Discussion

The result obtained from the pilot study of sodium chloride (NaCl) administered orally was 20.0 g/kg as LD100 for oral routes. According to the result, the LD100 indicates the least dose that caused 100% death of the experimental rats as shown in table 3.1.

In the other hand, LD50 of sodium chloride (NaCl) administered orally was 13.12 g/kg by the use of Karber's arithmetic method.

Therefore, according to (Matsumura, 1975; Elekima *et al*., 2017), LD50 rating of chemicals toxicity, sodium chloride given orally could be rated as practically non-toxic substance. The results obtained agree with the findings of Gaunt *et al*. (1967), which had 5.5 g/kg as LD50. In addition, as regards oral administration, LD50 of 10 g/kg was reported by Gaunt *et al*. (1967), when tartrazine was given orally which is in line with the findings of this work.

The discrepancy seen in the orally treated rats might be as result of the variation in the route of

administration. The discrepancy observed could be due to the interaction of the sodium chloride with intestinal content and chemical substances such as enzymes such as enzymes and activities of intestinal microorganisms that reduced the toxicity level before absorption into the systemic circulation. This report agreed with the findings in previous reports (Sasaki *et al*., 2002; EFSA, 2009; Elekima *et al*., 2017).

The results of the acute studies revealed as signs and symptoms of sodium chloride toxicity. The severity of the signs and symptoms were dosage dependent. In other words, as the dosages were increased, the more severity of the signs and symptoms of toxicity. This research agreed with the finding in previous reports (Gaunt *et al*., 1967; Matsumura, 1975; Ai-Mashhedy and Fijer, 2016; Elekima *et al*., 2017).

Conclusion

The LD100 of sodium chloride from the results obtained was 20.0 g/kg for oral route while the LD50 of sodium chloride (NaCl) administered orally was 13.12 g/kg. Therefore, the LD50 of sodium chloride given orally could be rated as practically non-toxic substance.

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