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DETERMINATION OF LD_{100} AND LD_{50} OF CARMIOSINE INTRAPERITONEALLY AND ORALLY ADMINISTERED IN ALBINO RATS

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ABSTRACT

The determination of LD_{100} and LD_{50} of carmiosine administered intraperitoneally and orally was carried out. A total of 68 albino rats weighing approximately 150g were used. In the determination of LD_{100} intraperitoneally, 10 rats were used with varying doses of 0.0g/kg, 0.17g/kg, 0.50g/kg, 1.0g/kg, 1.53g/kg, 2.0g/kg, 2.5g/kg, 3.33g/kg, 4.17g/kg, and 5.0g/kg while 10 rats were also used in the determination of LD_{100} orally with doses of 0.0g/kg, 5.0g/kg, 10.0g/kg, 12.5g/kg, 17.5g/kg, 22.5g/kg, 25.0g/kg, 32.5g/kg, 37.5g/kg and 40.0g/kg. In the acute study, rats were randomly separated into six groups. Each group had a total of four rats. The intraperitoneally treated groups were designated Aci, Bci, Cci, Dci, Eci and Fci with doses of 0.0g/kg, 0.17g/kg, 0.50g/kg, 1.0g/kg, 1.53g/kg, and 2.0g/kg respectively while that of oral were designated Aoc, Boc, Coc, Doc, Eoc and Foc were given doses of 0.0g/kg, 5.0g/kg, 10.0g/kg, 12.5g/kg, 17.5g/kg and 22.5g/kg respectively after LD_{100} dtermination. The LD_{50} of carmiosine given intraperitoneally and orally was calculated using the arithmetic method of karber. The LD_{100} was found to be 2.0g/kg and 22.5g/kg for intraperitoneally and orally treated rats respectively while the LD_{50} were 1.25g/kg and 13.37g/kg for intraperitoneal and orally route of administration. The LD_{50} obtained suggests that carmiosine is slightly toxic and practically non-toxic substance when administered intraperitonelly and orally respectively. The discrepancies seen in the intraperitoneal and orally treated rats could be due to the difference in the route of administration.

KEYWORDS: Carmiosine, toxicity, Pilot, Acute, LD_{100}, LD_{50}.

1. INTRODUCTION

Food dyes are broadly classified into natural and synthetic food dyes.[1,2] Carmosine is a synthetic dye that belongs to the azo class of the food dyes.[2,3] It is also called Azorubine S, Azorubine, food red 3 or acid red 14 with colour index of CI 14720.[4,5] Carmoisine dye impact reddish colouration on food or food products and it is seen in food products like yoghurts, breadcrumbs, cheesecake mixes, coloured cereals, rice, jellies and so on.[2,4]

Carmiosine toxicity has been reported in several studies inducing hepatic, renal and haematological derangements.[2,6,7,8,9] In 2012,[10] also reported that consumption of high doses of synthetic dyes such as carmiosine induced attention deficit disorder as well as hypersensitivity reactions. The toxicity of carmoisine is linked to the biotransformation process in the liver and actions of intestinal microorganisms producing aromatic amines, aryl amines and free radicals that attacks phospholipids bilayer of cell membranes especially inducing lesions and cell death.[2,11,12,13] In toxicological studies or toxicity rating of a chemical, it is important determine the LD_{100} and LD_{50} of the chemical. However, most toxicological research on synthetic food dyes such as carmiosine has failed to provide such information. Therefore, the aim of this work is to determine the LD_{100} and LD_{50} of carmiosine dye using albino rats. This work could provide the basic scientific knowledge on how LD_{100} and LD_{50} of chemical substances can be determined by other researchers.

2. MATERIALS AND METHODS

2.1 Materials

Materials used include weigh balance, gavage tube, carmiosine dye, sterile universal containers, 2ml & 5ml hypodermic syringes. Carmiosine dye with serial no FI19371 was obtained from Fiorio Colori Spa, Gessate, Italy in powdered form.

2.2 Experimental Animals

68 albino rats weighing 150g were used in this research work and were obtained through breeding in the Department of Medical Laboratory Science animal house

of Rivers State University, Port Harcourt. Twenty (20) rats were used for the pilot study while forty-eight (48) rats were used for the acute study. The rats were acclimatized for 10 days in a well-ventilated cage and were fed with pre-mix animal feed and water *ad libitum*.

2.3 Preparation of carmiosine dye (Pilot study)

Regarding intraperitoneal administration, 10.0g of the carmiosine was dissolved and mixed in 40.0ml of sterile sterile water. In other words, 1.0ml of the carmiosine solution contains 0.25g of carmiosine. However, in the oral route of administration, 15.0g of carmiosine dye was dissolved and mixed in 40.0 ml of sterile water. This also means that 1.0ml of the solution contains 0.375g of carmiosine.

2.4 Dosage and Administration (Pilot Study)

In the determination of LD_{100} of carmiosine given intraperitoneally, 10 rats were used with varying doses of 0.0g/kg, 0.17g/kg, 0.50g/kg, 1.0g/kg, 1.53g/kg, 2.0g/kg, 2.5g/kg, 3.33g/kg, 4.17g/kg, and 5.0g/kg while 10 rats were also used in the determination of LD_{100} of carmiosine given orally with doses of 0.0g/kg, 5.0g/kg, 10.0g/kg, 12.5g/kg, 17.5g/kg, 22.5g/kg, 25.0g/kg, 32.5g/kg, 37.5g/kg and 40.0g/kg.

2.5 Acute Toxicity Studies

Rats for acute toxicity studies were grouped into six. Each group had a total of four rats. The intraperitoneally treated groups were designated Aci, Bci, Cci, Dci, Eci and Fci and were treated with 0.0g/kg, 0.17g/kg, 0.50g/kg, 1.0g/kg, 1.53g/kg and 2.0g/kg doses of carmiosine respectively while that of oral group were designated Aoc, Boc, Coc, Doc, Eoc and Foc and were also treated with 0.0g/kg, 5.0g/kg, 10.0g/kg, 12.5g/kg, 17.5g/kg and 22.5g/kg doses of carmiosine respectively after LD_{100} determination.

2.6. Determination of LD_{50} of carmiosine dye

The LD_{50} for intraperitoneal and oral treatments were obtained using the Arithmetic Method of Karber after establishing the LD_{100} from the pilot toxicity studies. The arithmetic method of karber is expressed as:

$$LD_{50} = LD_{100} - \left(\frac{\text{Sum of dose diff. x Mean dead}}{\text{No of rats}}\right)$$

3. RESULTS

In the pilot study, after the treatment, the treated rats were monitored within 24 hours for signs and symptoms of carmiosine toxicity such as pigmentation, sedation, respiratory distress and coma were observed until death occurred. From the pilot toxicity studies, 1.2mls and 9.0mls of carmiosine given intraperitneally and orally caused 100% death (LD_{100}) in the animals which were calculated to be 2.9g/kg and 22.5g/kg respectively (Table 3.1 and Table 3.2).

Table 3.1: Determination of Minimum Dose that Caused 100% Deaths (LD_{100}) of Carmiosine Intraperitoneally Treated Rats.

Groups	No of rat	Volume (ml)	Dose (g/kg)	Alive?	Dead?
1	1	0.0	0.0	YES	NO
2	1	0.10	0.17	YES	NO
3	1	0.30	0.50	YES	NO
4	1	0.60	1.0	YES	NO
5	1	0.90	1.53	YES	NO
*6	1	*1.20	*2.0	NO	YES
7	1	1.50	2.5	NO	YES
8	1	2.0	3.33	NO	YES
9	1	2.50	4.17	NO	YES
10	1	3.0	5.0	NO	YES

*The minimum dose that caused 100% death (LD_{100}) in the rats.

Table 3.2: Determination of Minimum Dose that Caused 100% Deaths (LD_{100}) of Carmiosine Orally Treated Rats.

Groups	No of rat	Volume (ml)	Dose (g/kg)	Alive?	Dead?
1	1	0.0	0.0	YES	NO
2	1	2. 0	5.0	YES	NO
3	1	4.0	10.0	YES	NO
4	1	5. 0	12.5	YES	NO
5	1	7.0	17.5	YES	NO
*6	1	*9.0	*22.5	NO	YES
7	1	10.0	25.0	NO	YES
8	1	13.0	32.5	NO	YES
9	1	15.0	37.5	NO	YES
10	1	16.0	40.0	NO	YES

*The minimum dose that caused 100% death (LD_{100}) in the rats.

In the acute toxicity studies, signs and symptoms of carmiosine toxicity like pigmentation, sedation, respiratory distress, coma and death were observed in the intraperitoneally treated rats. It group was noted that death occurred at Dci, Eci and Fci with an average time of 6.0, 5.5 and 4.7 hours respectively (Table 3.3). The arithmetic method of Karber was used to determine the LD_{50}. Applying the arithmetic method of Karber, the LD_{100} = 2.0g/kg, sum of dose difference × mean dead =3.0, number of rats per group = 4.0. Therefore, LD_{50} = 2.0 – (3/4.0) = 2.0 -0.75 = 1.25g/kg (Table 3.4). Based on [14], rating of chemical toxicity, carmiosine with an LD_{50} of 1.25g/kg administered intraperitoneally is slightly toxic.

Table 3.3: Acute Toxicity Study of Carmiosine Intraperitoneally Administered and average time of death.

Groups	Dose (g/kg)	No of rats	No of death	No alive	Av. time of death (Hr)
A_{CI}	0.00	4	0	4	
B_{CI}	0.17	4	0	4	
C_{CI}	0.5	4	0	4	
D_{CI}	1.0	4	2	2	6.0
E_{CI}	1.5	4	2	2	5.5
F_{CI}	2.0	4	4	0	4.7

Hr = Hour

Table 3.4.: Determination of Median Lethal Dose (LD_{50}) for Carmiosine Intraperitoneally Treated Rats.

Groups	Dose (g/kg)	Dose diff	No dead	Mean dead	Dose diff × Mean death
A_{CI}	0.00	0.0	0	-	-
B_{CI}	0.17	0.17	0	-	-
C_{CI}	0.5	0.33	0	-	-
D_{CI}	1.0	0.5	2	1.0	0.5
E_{CI}	1.5	0.5	2	2.0	1.0
F_{CI}	2.0	0.5	4	3.0	1.5
				Total	3.0

Regarding the oral treatment, signs and symptoms of carmiosine toxicity like pigmentation, sedation, respiratory distress, coma and death were also observed. It was seen that death occurred at group Doc, Eoc and Foc with an average time of 7.0, 6.2 and 4.9 hours respectively (table 3.5). The LD_{50} was also determined using the arithmetic method of Karber. Applying the arithmetic method of karber, the LD_{100} = 22.5g/kg, sum of dose difference × mean dead = 36.5, number of rats per group = 4.0. Therefore, LD_{50} = 22.5 – (36.5/4.0) = 22.5 – 9.13 = 13.37g/kg. Based on [14], rating of chemical toxicity, carmiosine with LD_{50} of 13.37g/kg administered orally is practically non-toxic.

Table 3.5: Acute Toxicity Study of Carmiosine Orally Administered and average time of death.

Groups	Dose (g/kg)	No of rats	No of death	No alive	Av. time of death (Hr)
A_{OC}	0.00	4	0	4	
B_{OC}	5.0	4	0	4	
C_{OC}	10.0	4	1	3	
D_{OC}	12.5	4	2	2	7.0
E_{OC}	17.5	4	3	1	6.2
F_{OC}	22.5	4	4	0	4.9

Hr =Hour

Table 3.6: Determination of Median Lethal Dose (LD_{50}) for Carmiosine Orally Treated Rats.

Groups	Dose (g/kg)	Dose diff	No dead	Mean dead	Dose diff × Mean death
A_{OC}	0.00	0.0	0	-	-
B_{OC}	5.0	5.0	0	-	-
C_{OC}	10.0	5.0	1	0.5	2.5
D_{OC}	12.5	2.5	2	1.5	3.8
E_{OC}	17.5	5.0	3	2.5	12.5
F_{OC}	22.5	5.0	4	3.5	17.5
				Total	36.5

4. DISCUSSION

According to the results obtained from the pilot study, the LD_{100} of carmiosine were 2.0g/kg and 22.5g/kg for intraperitoneal and oral routes of administration respectively (Table 3.1 and Table 3.2). Also, the LD_{50} of carmiosine given intraperitoneally and orally were 1.25g/kg and 13.43g/kg respectively using the Arithmetic Method of Karber (Table 3.4 and Table 3.6). [14], rating of chemicals toxicity, carmiosine administered intraperitoneally could be rated as slightly toxic substance but when administered orally, it is a practically non-toxic substance. This result is in agreement with the reports of[15,16] which had LD_{50} of 1.0g/kg for carmiosine intraperitoneally administered. In terms of oral administration, the result obtained is in line with the report of,[15] who reported LD_{50} of $\geq$10.0g/kg.

More so, the acute studies also showed signs and symptoms of carmiosine toxicity such as pigmentation, sedation, respiratory distress, coma and death. The severity of these signs and symptoms of toxicity were dosage dependent. That is, as the doses were increased the more severe the signs and symptoms of toxicity. As shown in table 3.3 and table 3.5 of the acute toxicity studies, the signs and symptoms of carmiosine toxicity such as the time of death (hours) in the intraperitoneally treated rats were shorter compared to the oral route of administration. These findings agree with the reports of.[16]

The significant differences seen in the LD_{100} and LD_{50} of the intraperitoneally and orally treated rats are possibly due to the route of administration. With respect to the oral route of administration, prior to systemic absorption, there could have been interaction of the carmiosine dye with intestinal secretions, enzymes and microbial actions which probably have reduced the degree of toxicity compared to intraperitoneal route administration were absorption to systemic circulation is without much interference. In addition, because of the ease in the excretion of un-absorbed dyes via faeces, the oral route of administration tends to impact less toxicity (the smaller the value of the LD_{50}, the more toxic the substance and the larger the LD_{50} value the lower the toxicity) compared to intraperitoneal route of administration. Our findings is also in line with the reports of.[11,13,14,15]

5. CONCLUSION

From the studies, the LD_{100} of carmiosine administered intraperitneally and orally were 2.9g/kg and 22.5g/kg while the LD_{50} were 1.25g/kg and 13.37g/kg respectively. Based on the rating of chemical toxicity, carmiosine with an LD_{50} of 1.25g/kg administered intraperitoneally is slightly toxic while the LD_{50} of 13.37g/kg when administered orally is practically non-toxic.

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