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PILOT AND ACUTE TOXICITY STUDIES ON TARTRAZINE IN ALBINO RATS

***[1]Elekima Ibioku, [2]Nwachuku Edna Ogechi and [3]Nduka Nsirim**

[1,2,3]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

***Corresponding Author: Elekima, Ibioku**

Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

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ABSTRACT

Pilot and acute toxicity studies were carried out to determine the LD_{100} and LD_{50} of tartrazine administered intraperitoneally and orally. A total of 68 albino rats weighing approximately 150g were used. 10 rats were used for the determination of LD_{100} of tartrazine administered intraperitoneally with doses of 0.0g/kg, 1.68g/kg, 3.33g/kg, 5.0g/kg, 6.67g/kg, 8.33g/kg, 10.0g/kg, 13.33g/kg, 15.0g/kg and 16.67g/kg. Likewise, 10 rats were also used in the determination of LD_{100} of tartrazine administered orally with doses of 0.0g/kg, 2.5g/kg, 5.0g/kg, 10.0g/kg, 15.0g/kg, 20.0g/kg, 22.5g/kg, 30.0g/kg, 35.0g/kg and 40.0g/kg. In the acute study, rats for intraperitoneal and oral administration were classified into six groups. Each group had a total of four rats. The intraperitoneally treated groups were designated Ai, Bi, Ci, Di, Ei and Fi and were treated with 0.0g/kg, 1.68g/kg, 3.33g/kg, 5.0g/kg, 6.67g/kg, 8.33g/kg respectively while that of oral were designated Ao, Bo, Co, Do, Eo and Fo were also treated with 0.0g/kg, 2.5g/kg, 5.0g/kg, 10.0g/kg, 15.0g/kg, 20.0g/kg respectively after LD_{100} determination. From studies, the LD_{100} were found to be 8.33g/kg and 20.0g/kg while the LD_{50} were found to 5.83g/kg and 11.25g/kg for intraperitoneally and orally treated rats respectively. Based on the LD_{50} obtained, tartrazine could be classified as practically non-toxic substance according Matsumura toxicity rating of chemical when administered intraperitoneally or orally.

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

1. INTRODUCTION

Colours are distinct components of food and food products that stimulates consumers' satisfaction.[1][2] Tartrazine is a synthetic food dye derived from coal-tar and it is widely applied in food, pharmaceutical and cosmetic companies.[2][3][4] Ingestion of tartrazine in food products have also been linked to cause derangements such as anaemia, renal and hepato-toxicity, leucopenia and so on in rats.[3][5][6][7][8][9] In humans, especially children, it has been reported to cause attention deficit disorders and allergic reactions.[2][10]

Toxicity of tartrazine as reported in several studies has been linked to oxidative stress induced by the generated aromatic amine, aryl amine, reactive hydroxyl group and superoxides viz-a-viz azo dye metabolism by the liver and microbes of the intestine[11][12]

In toxicity study, the establishment of LD_{100} and LD_{50} in order to rate the substance of interest is very crucial. However, most of the studies on toxicity of tartrazine did not take that into consideration. Therefore, the aim of this research is to determine the LD_{100} and LD_{50} of tartrazine for both intraperitoneal and oral route of administration through pilot and acute toxicity studies. The pilot toxicity study is used to establish the minimum dose that cause 100% deaths (LD_{100}) while the acute toxicity study provide the minimum lethal dose that killed 50% (LD_{50}) of the study animal.[13]

2. MATERIALS AND METHODS

2.1 Materials

Materials used in this study include gavage tube, 2ml & 5ml hypodermic syringes, tartrazine food dye which was purchased dye in granular form with serial no FI19371 from Fiorio Colori Spa, Gessate, Italy.

2.2 Experimental Animals

A total 68 albino rats weighing approximately 150g were used in this study. The albino rats used in this study were acquired through breeding in the Department of Medical Laboratory Science animal house of Rivers State University, Port Harcourt. Feeding of the animals were achieved using rat pre-mix feed and water *ad libitum* and were housed in a well ventilated cages with the water cans and fed containers in position.

2.3 Preparation of tartrazine dye for pilot and acute toxicity studies

For intraperitoneal administration, 10.0g of the tartrazine was dissolved in 40.0mls of sterile water. This connotes that 1.0ml of the solution contains 0.25g of tartrazine. In

the oral route of administration, 15.0g of the tartrazine was also dissolved 40.0mls of sterile water. This also connotes that 1.0ml of the solution contains 0.375g of tartrazine.

2.4 Dosages and Administration of tartrazine dye for pilot toxicity studies

10 rats were used for the determination of LD_{100} of tartrazine administered intraperitoneally in the pilot study. The doses were 0.0g/kg, 1.68g/kg, 3.33g/kg, 5.0g/kg, 6.67g/kg, 8.33g/kg, 10.0g/kg, 13.33g/kg, 15.0g/kg and 16.67g/kg. Likewise, 10 rats were also used in the determination of LD_{100} of tartrazine administered orally. The doses were 0.0g/kg, 2.5g/kg, 5.0g/kg, 10.0g/kg, 15.0g/kg, 20.0g/kg, 22.5g/kg), 30.0g/kg, 35.0g/kg and 40.0g/kg.

2.5 Acute Toxicity Studies: Dosages

Rats for acute toxicity studies in both oral and intraperitoneally treated rats were classified into six groups. Each group had a total of four rats. The intraperitoneally treated groups were designated Ai, Bi, Ci, Di, Ei and Fi while that of oral were designated Ao, Bo, Co, Do, Eo and Fo respectively. Group Ai, Bi, Ci, Di, Ei and Fi were administered with 0.0g/kg, 1.68g/kg, 3.33g/kg, 5.0g/kg, 6.67g/kg, 8.33g/kg after determination of the LD_{100} of the pilot studies while group Ao, Bo, Co, Do, Eo and Fo were given doses of 0.0g/kg, 2.5g/kg, 5.0g/kg, 10.0g/kg, 15.0g/kg, 20.0g/kg respectively after LD100 determination as well.

2.6. Determination of LD_{50} of tartrazine

The LD_{50} of tartrazine administered intraperitoneally and orally was obtained using the arithmetic method of karber after determing the LD_{100} from the pilot toxicity studies in both intraperitoneally and orally treated rats respectively. The arithmetic method of karber for calculating LD_{50} was given as follows.

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

3. RESULTS

After the administration of the dye in the pilot study, the treated rats were monitored within 24 hours for signs and symptoms of tartrazine toxicity such as pigmentation, sedation, respiratory distress, coma until death occurred. The minimum dose that caused 100% death was seen as the LD_{100}.

From the pilot toxicity studies carried out, the minimum dose that caused 100% death (LD_{100}) in the animals was 8.33g/kg and 20.0g/kg for intraperitoneally and orally treated rats respectively (Table 3.1 and Table 3.2).

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

Groups	No of rat	Volume (ml)	Dose (g/kg)	Alive?	Dead?
1	1	0.0	0.0	YES	NO
2	1	1.0	1.67	YES	NO
3	1	2.0	3.33	YES	NO
4	1	3.0	5.00	YES	NO
5	1	4.0	6.67	YES	NO
*6	1	*5.0	*8.33	NO	YES
7	1	6.0	10.0	NO	YES
8	1	8.0	13.33	NO	YES
9	1	9.0	15.0	NO	YES
10	1	10.0	16.67	NO	YES

* Minimum Dose that Caused 100% Death (LD_{100})

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

Groups	No of rat	Volume (ml)	Dose (g/kg)	Alive?	Dead?
1	1	0.0	0.0	YES	NO
2	1	1.0	2.5	YES	NO
3	1	2.0	5.0	YES	NO
4	1	4.0	10.0	YES	NO
5	1	6.0	15.01	YES	NO
*6	1	*8.0	*20.0	NO	YES
7	1	9.0	22.5	NO	YES
8	1	12.0	30.0	NO	YES
9	1	14.0	35.0	NO	YES
10	1	16.0	40.0	NO	YES

* Minimum Dose that Caused 100% Death (LD_{100})

The LD_{50} for intraperitoneally treated rats were determined using the arithmetic method of karber after LD_{100} determination. Signs and symptoms of tartrazine toxicity such as pigmentation, sedation, respiratory distress, coma and death were observed. It was shown that death occurred at group Di, Ei and Fi with an average time of death of 5.3, 4.7 and 3.7 hours respectively (Table 3.3). Therefore, applying the Arithmetic Method of Karber, the LD_{100} is 8.33g/kg, sum of dose difference × mean dead =9.99, number of rats per group = 4.0. Therefore, LD_{50} = 8.33 – (9.99/4.0) = 8.33 – 2.50 = 5.83g/kg (Table 3.4). Based on[13], rating of chemical toxicity, tartrazine with an LD_{50} of 5.83g/kg administered intraperitoneally is practically non-toxic.

Table 3.3: Acute Toxicity Study of Tartarzine Intraperitoneally Administered in Rats with average time of death.

Groups	Dose (g/kg)	No of rats	No of death	No alive	Av. time of death (Hr)
A_{TIP}	0.00	4	0	4	
B_{TIP}	1.67	4	0	4	
C_{TIP}	3.33	4	0	4	
D_{TIP}	5.0	4	1	3	5.3
E_{TIP}	6.67	4	3	1	4.7
F_{TIP}	8.33	4	4	0	3.6

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

Groups	Dose (g/kg)	Dose diff	No dead	Mean dead	Dose diff × Mean death
A_{TIP}	0.00	0.00	0	-	-
B_{TIP}	1.67	1.67	0	-	-
C_{TIP}	3.33	1.66	0	-	-
D_{TIP}	5.0	1.67	1	0.5	0.84
E_{TIP}	6.67	1.67	3	2.0	3.34
F_{TIP}	8.33	1.66	4	3.5	5.81
				Total	9.99

In terms of LD_{50} for orally treated rats, after the determination of LD_{100} for orally treated rats, the LD_{50} for orally treated rats were also determined using the arithmetic method of karber. Signs and symptoms of tartrazine toxicity like pigmentation, sedation, respiratory distress, coma and death were observed. It was shown that death occurred at group Do, Eo and Fo with an average time death of death of 5.9, 4.9 and 3.8 hours respectively (Table 3.5). Therefore, applying the arithmetic method of karber, the Minimum dose that caused 100% death (LD_{100}) = 20.0, sum of dose difference × mean dead = 35.0, number of rats per group = 4.0. Therefore, LD_{50} = 20.0 – (35.0/4.0) = 20.0 – 8.75 = 11.25g/kg (Table 3.6). Based on[13] rating of chemical toxicity, tartrazine with an LD_{50} of 11.25g/kg administered orally is practically non-toxic.

Table 3.5: Acute Toxicity Study of Tartrazine Orally Administered in Rats with average time of death.

Groups	Dose (g/kg)	No of rats	No of death	No alive	Av. time of death (Hr)
A_{TO}	0.00	4	0	4	
B_{TO}	2.5	4	0	4	
C_{TO}	5.0	4	0	4	
D_{TO}	10.0	4	2	2	5.9
E_{TO}	15.0	4	3	1	4.9
F_{TO}	20.0	4	4	0	3.8

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

Groups	Dose (g/kg)	Dose diff	No dead	Mean dead	Dose diff × Mean death
A_{TO}	0.00	0.0	0	-	-
B_{TO}	2.5	2.50	0	-	-
C_{TO}	5.0	2.50	0	-	-
D_{TO}	10.0	5.0	2	1	5.0
E_{TO}	15.0	5.0	3	2.50	12.5
F_{TO}	20.0	5.0	4	3.50	17.5
				Total	35.0

4. DISCUSSION

From the pilot study carried out, the results obtained were 8.33g/kg and 20.0g/kg as LD_{100} for intraperitoneal and oral routes respectively. According to the results, the LD_{100} indicate the least dose that caused 100% death of the experimental rats as shown in table 3.1 and table 3.2. Also, LD_{50} of tartrazine administered intraperitoneally and orally were 5.83g/kg and 11.25g/kg respectively by the use of Arithmetic Method of Karber (Table 3.4 and Table 3.6). Furthermore, as shown in the acute results, time of death (hours) in intraperitoneally treated rats were shorter compared to the oral route of administration.

According to[13], LD_{50} rating of chemicals toxicity, tartrazine given intraperitoneally or orally could be rated as practically non-toxic substance. The results obtained agree with the findings of[14], which had 3.8g/kg and 5.5g/kg as LD_{50} while[5][16][17], had LD_{50} of >2.0g/kg for tartrazine intraperitoneally administered. In addition, as regards oral administration, LD_{50} of 10g/kg was report by[14], when tartrazine was given orally which is in line with the findings of this work.

The discrepancies seen in the intraperitoneally and orally treated rats might be as result of the variation in the route of administration. Regarding the oral route as compared to the intraperitoneal, the discrepancies observed could be due to the interaction of the dye with intestinal content and chemical substances such as enzymes as well as the activities of intestinal micro-organisms that reduced the toxicity level before absorption into the systemic circulation. Our findings concur with the reports of.[15][16]

The results of the acute studies revealed as signs and symptoms of tartrazine 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 report is in agreement with the finding of.[13][14][17]

5. CONCLUSION

The LD_{100} of tartrazine from the results obtained were 8.33g/kg and 20.0g/kg for intraperitoneal and oral routes respectively while the LD_{50} of tartrazine administered intraperitoneally and orally were 5.83g/kg and 11.25g/kg respectively. Therefore, the LD_{50} of tartrazine given intraperitoneally or orally could be rated as practically non-toxic substance.

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