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Acute Toxicity of Food additives Tartrazine and carmoisine on white male Mice

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Abstract: *This study* was conducted to evaluate the acute toxicity by measures median lethal dose of two different types of Food additives; tartrazine (E102) and carmoisine (E122) in the white male mice. In this study different doses of each dye (1250, 2500, 3750, 5000 and 6250) mg/Kg BW was administered orally by stomach tube to the different groups of experimental animals. The signs of toxicity and possible death of mice for each group were monitored and calculated through 3 days to determine LD50. The present investigations reveal that, the LD50 value of the carmoisine was 4166.66 mg/kg BW. the clinical signs of toxicity was loss of appetite, drowsiness, tachycardia, decrease in locomotion & anorexia & according to the Hodge and sterner toxicity scale the substance consider slightly toxic whereas the LD50 of tartrazine was found to be more than 6250 mg /Kg BW & no mortality rate was observed after single dose administration in all groups and according to the Hodge & sterner scale the substance classified as practically non toxic.
Keywords : Acute toxicity ,tartrazine ,carmoisine ,mice.

Introduction:

Food additives have an important role to meet the needs of population growth[(1)]. Food additives can be either artificial or natural foodstuffs and food coloring materials can be used[(2)]. Food additives Give food colorings aesthetic appearance of the materials desired by consumers juices.[(3)] azo dyes have a wide range of applications in the textile ,leather, paper ,food, pharmaceutical and cosmetics[(4)] azo colors are characterized by the existence of group(N = N) linked to the aromatic rings in the molecular structures[(5-9)]. Food colorings a startrazine (E102) carmoisine (E122),.Tartrazineis one of AZO dyes and it provides alemon yellow color. Most of the food and some non-food products include tartrazine colorant such as chips, soft drinks, sweets, ice cream, soap, shampoo, medical capsules and medicines is soluble in water to give a yellow colour[(3)] the chemical structure of tartrazine is is sodium (E) -5-oxo -1-(4-sulfonatophenyl) -4 - ((4-sulfonatophenyl) diazenyl) -4, 5-Hydro -1 H-pyrazole-3-carboxylate (C16 H9 N4 Na3 O9 S2). Carmoisine contains a (–N=N) group unilateral too. It is usedtintedredfromvarious industriesdyes, E numberis toCarmoisineE122[(5)]. It givescolorred whenit solvedin water.

The chemical composition ofdisodium 4-hydroxy-3- (4-sulfonat-1-naphthylazo) naphthalene-1-sulphonate (chemical formula C20H12N2Na2O7S2) .Azo dyes can be reduced to aromatic amines by intestinal microflora and perhaps by mammalian azoreductase in the liver or in the intestinal wall following by ingestion [(11)]. If the azo dyes are reduced completely to aromatic amines, P450 enzymes oxidize, these aromatic amines are N-hydroxy derivatives [(11)]. the prolonged use various types of azo dyes can cause certain diseases such as anemia, indigestion, and lesions in the brain, liver, kidney, spleen, tumors and cancer, and the lack of growth, lack of mental, abnormalities in the springs off and disadvantages of the eye leading to blindness and allergic

reactions and rashes nettle rash, asthma and lead to increase oxidative stress[3]Toxicity studies are Classified into, acute toxicity, sub-acute toxicity and chronic toxicity studies[12].

LD50 determination is a simple preliminary assessment of the toxicity of a compound by determination of the mean lethal dose which is capable of death 50% of animals under declared conditions [7]. In terms of signs or symptoms, LD50 is redefined as the dose active for producing a certain mark in 50% of the experimental animals. So the present study was aimed to evaluate acute toxicity for two different food additives (tartrazine & carmoisine) in experimental mice.

Materials and methods:

Test chemicals:

Tartrazine and carmoisine were obtained from Sigma Aldrich Company. All glassware's which used in this study were properly washed with distilled water and oven dried before use.

Experimental animals:

72 mature male Sprague- Dawly white mice of 8-9 weeks old and weighing between 25-30 gm, were obtained from the college of science at the University of Babylon. All the animals were allowed to acclimatize to the experimental conditions for a period of 7 days. The animals were housed in polyacrylic cages, not more than three animals per cage under standard laboratory condition relative humidity at 50-55%, room temperature 22±2C° and 12 hrs. Light: dark cycle. The animals were fed with standard diet and water *ad libitum*, except at the period of experiment. Before the beginning of experiment, mice were placed on a raised wire mesh, under a clear plastic box and allowed two hours to acclimate to testing room.

Experimental procedure:

The acute toxicity test was conducted to determine the oral LD50 of two different food additives separately. The male white mice were randomly divided into tartrazine group & carmoisine group. each group divided to 6 subgroups of 6 animals were received orally 5 different dosages of each dye alone as following (1250 mg/kg ,2500 mg/kg ,3750 mg/kg ,5000 mg/kg , 6250 mg/kg) while mice in the control group received distilled water . The mice were observed for 3 days for the signs and symptoms of toxicity as well as the death rate of each group were recorded.

Calculation of LD50:

The LD50 of the substances was calculated using the arithmetic method of Karber as modified by [13].

The LD50 was calculated using the following formula:

LD50 =LDy – Σ (Dd x md)/N
Where LDy =Highest dose (LD100)
N =Number of animals per group
Dd =Dose difference
Md =Mean dead
LD50 =Dose that killed 50% of experimental animals
LD100 = Dose that killed 100% (all) the experimental animals

Results and discussion:

The determination of LD50 value is one of the salient methods used in acute toxicity studies and it represent the dose that kills fifty percent of the experimental group animals. LD50 values obtained also depend on the route of administration of the drug. Usually the values are found to increase with the following sequence of routes: intravenous, intraperitoneal, subcutaneous and oral [14].The oral route of administration was used in the present study to determined the acute toxicity of two different food additives dyes used commonly for preparation of various products. In the animals that received different doses of the carmoisine dye ranging from (1250 to 6250 mg/Kg) administered orally in a single dose by stomach tube, the signs of loss of appetite,

drowsiness, tachycardia, decrease in locomotion & anorexia was distinctive signs observed on the mice before dead. The present investigations reveal that, the LD50 value of the carmoisine dye was 4166.66 mg/kg BW, therefore according to the Hodge and sterner toxicity scale the carmoisine considered slightly toxic substance.[15].table(1). JECFA mentions acute toxicity studies of carmoisine dye in rats and mice as experimental design. In these studies, LD50 values were found to be > 8000 and > 10000 mg/kg/BW, respectively. In addition, acute toxicity tests were conducted with intraperitoneal and intravenous administration, but these studies are considered to be of little relevance for the toxicological evaluation of food additives [16] , also was determined the acute toxicity of carmoisine in rats & mice by two different route of administration[12]. The acute intraperitoneal LD50 was approximately 1 g/kg in mice and rats. Orally, doses up to 8 g/kg in mice and 10 g/kg in rats were tolerated without lethal effect. On the other hand from the experiment, the results of the present study reveal that the tartrazine dye administration at single dose has not been found to be any toxic effects even at higher dose used (6250 mg/Kg BW) at the same time there is no mortality or morbidity was recorded in all grouped animals treated with tartrazine dye, So according to the Hodge & sterner scale the substance classified as practically non toxic. Several short-term and sub-chronic toxicity studies on tratrazine dye was done in rats, cats and dogs and reviewed by [17]. No tartrazine-related effects were reported for doses up to 500 mg/kg BW. Tartrazine was recorded to induce neurological effects and deficits in learning and memory in animals [17,18] at doses in excess of the acceptable daily intake of tartrazine (0-7.5 mg/kg/day). However, it could not be excluded that exposure to tartrazine together with other dyes exerted toxicity by mechanisms involving synergistic process.

Table (1) Determination of LD50 value of dyes in mice

Dose of substance	No of mice in group	No. of death	Mean death	Dose difference	Mean death ×Dose diff.
1250mg/kg	6	0	0	0	0
2500mg/Kg	6	0	0	1250	0
3750/mg/Kg	6	2	1	1250	1250
5000mg/kg	6	5	3.5	1250	4375
6250mg/kg	6	6	5.5	1250	6875
LD50 = LDy-Σ(Dd×md) /N = 6250 -12500/6 = 4166.66					12500

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