EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 2
85-90
Year: 2020

EFFECT OF FOOD ADDITIVES; CARMOISINE AND ACESULFAME POTASSIUM ON THE HEART OF ADULT WISTAR RAT

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Article Received on 15/12/2019 **Article Revised on 05/01/2020** **Article Accepted on 26/01/2020**

ABSTRACT
Carmoisine and acesulfame potassium are widely used as food additives. This study examined the effect(s) of oral administration of carmoisine and acesulfame potassium on the histology of the heart. Twenty adult wistar rats weighing between 130-180g were divided into five groups of four animals each. Group A (control) was given rat feed and water; group B received 500mg/kg body weight of carmoisine, group C received 500mg/kg body weight of acesulfame potassium while group D was co-administered 250mg/kg body weight of each of carmoisine and acesulfame potassium. All food additives were given daily by oral gavage method for twenty-one days. Twenty-four hours after the last administration, the rats were sacrificed. The hearts were harvested, weighed and fixed in 10% formol saline for histological studies. Our result showed a significant increase in the relative heart weight in group B, compared to the control group. Histological observation showed that following administration of carmoisine and acesulfame potassium, there was mild infiltration of heart tissue by inflammatory cells. The result of the study revealed that exposure of rats to carmoisine and acesulfame pottassium at these concentrations caused mild distortion of cardiac fibers which may constitute a risk factor for cardiac function.

KEYWORDS: Carmoisine, Acesulfame Potassium, Heart, food additive.

INTRODUCTION
Food additives (either food dyes or sweeteners) are chemicals added to foods with the aim of improving and enhancing their flavour, taste, colour, texture and for preservation (Downham. and Collins 2000). In making the food products, many attractive types of natural and synthetic dyes were used. Comparatively, the synthetic food dyes are stable, less expensive and occupy an important place in food industry (Nayak and Nath 2007). Food additives have extremely important role to meet the needs of the growing population during production and presentation of plentiful, tasty and nutritious food (Gao *et al.*, 2011). Additives are present in little quantities in foods but have great impact as many of them contain toxic components which when consumed repeatedly may result in alteration of normal cellular functions and induction of pathophysiological conditions such as cancer, atherosclerosis, diabetes (Soltan and Manal 2012). Many currently approved food dyes have been banned in some countries due to their adverse effects on laboratory animals and human (Cook, 2013). This notwithstanding, many poor countries still have it smuggled into the markets and they still find their way into our cooking pots, especially for commercial food vendors.

The effects of food additives may be immediate or may be harmful in the long run if one has constant exposure or accumulations. Immediate effects may include headaches, change in energy level, and alterations in mental concentration, behaviour, or immune response (Soltan and Manal 2012). Long-term effects may increase one's risk of cancer, cardiovascular disease and other degenerative conditions. Some modern synthetic preservatives have become controversial because they have been shown to cause respiratory or other health problems (Soltan and Manal 2012). Some studies point to synthetic preservatives and artificial colouring agents aggravating ADD & ADHD symptoms in those affected (Gustafsson *et al*., 2003). Parental reports were more accurate indicators of the presence of additives than clinical tests (ANON 2004). Allergic preservatives in food or medicine can cause an anaphylactic shock in susceptible individuals, a condition which is often fatal within minutes without emergency treatment. It is almost a certainty that few really know what it is, that is part of their foodstuffs, and yet may present threats and danger.

Essentially, there are two main sources of dangerous or threatening additives. The first is those that are put in as part of the processing operation. These include the colourings, preservatives, flavours and flavour enhancers, sweeteners, texture agents and processing agents.

The second source of additives to our food is from packaging, storing and handling of food and these informations are not normally included on the label of the food. Food that has no additives at all is to be preferred, most especially if it is to be used to feed children. Many foods available in the market contain different types of preservatives. These chemicals can give rise to certain health problems (Abdulmumeen *et al.*, 2012).

Azorubine also known as carmoisine is a synthetic azo dye most widely used in heat-treated foods, condiments, candy, baked food products, ice cream and mouthwash. carmoisine causes allergic or intolerance reactions particularly in individuals with aspirin intolerance. Other reactions may include rash similar to nettle rash and skin swelling (Ford *et al.*, 1987). Azo dyes have been reported to be carcinogenic and linked to bladder cancer (Golka *et al.*, 2012).

Acesulfame potassium is one of the non-nutritive sweeteners that aids patients with type 1 diabetes. It provides a very sweet taste without affecting glycaemic responses and without the high content of caloric sugars. Some studies, however, discovered that the consumption of non-nutritive sweeteners has led to weight gain thus increasing the risk of type 2 diabetes (Dewinter *et al.*, 2015).

MATERIALS AND METHODS

MATERIALS

Dissecting kit, Mettle teledo weighing machine (Monobloc weighing technology 1118380934, 2012), Electronic weighing balance (Leica CT 250 1101735428, 2012), Absorbent cotton wool, 10% formol saline, Haematoxylin and eosin (H and E), Dissecting pins/optical pins normal, Dash board, 20 adult Wistar rats, Perpex cages, Drinkers (plastic), Pyrex glass Beaker (100ml, 250ml and 500ml capacity), Measuring cynlinder (100ml capacity), Distilled water, Plates (for feeding) and Saw dust/wood sharing.

EXPERIMENTAL PROCEDURE

Substance Of Study

Azorubine also known as carmoisine (TCI chemicals PVT limited India, CAS Number: 3567-69-9, Product number: A0580) and Acesulfame potassium (TCI chemicals PVT limited India, CAS Number: 55589-62-9, Product number: A1490) were obtained from Dubem Chemical store at Head Bridge Market Onitsha.

Experimental Animals

A total of 20 adult wistar rats were used for the experiment. They were obtained from the Department of Physiology, Nnamdi Azikiwe University, Nnewi Campus and were housed in the Central Animal House, Nnamdi Azikiwe University College of Health Sciences Nnewi Campus. Standard ethical procedures were observed in animal handling. The animals weighed from 130g-180g. The rats were kept in wooden cages at optimum temperature, 12hrs light/dark cycle and fed with commercial grower smash from Top feeds and water *ad libitum*. Each of the rats was identified using non-invasive method (permanent markers of different colors). The experiment was carried out in accordance with current rules and guidelines established for care of laboratory animals.

Experimental Design

The experiment lasted for a period of five (5) weeks; two (2) weeks for acclimatization and three (3) weeks for administration of food additives (carmoisine and acesulfame potassium). Animals were randomly grouped and treated as follows;

Group A; Served as control that receives normal feed and water

Group B; received 500mg/kg body weight carmoisine,

Group C; received 500mg/kg body weight acesulfame potassium

Group D; was co-administered 250mg/kg body weight each of carmoisine and acesulfame potassium.

At the end of the 5 weeks, the rats were sacrificed by cervical dislocation and the heart were weighed and immediately fixed in 10% formol saline in a labeled container specific for each rat.

Histological Evaluation

After 72 hours of fixing, the tissues were immersed into graded alcohols: 70%, 80%, 90%, 95% and absolute for 1 hour each to ensure proper dehydration. On removal of the tissues from absolute alcohol, the tissues were passed through 2 changes of xylene (I, II) for 1 hour each. On removal of the tissue from the clearing agent, they were immersed in 2 changes of wax bath for 2 hours each in a hot air oven. Infiltration was done in molten paraffin wax at a temperature of 60°C for two hours each in two changes. The embedded tissues were sectioned with a rotary microtome. Haematoxylin and eosin method was used for staining after sectioning.

Statistical Analysis

The result of the data was statistically analyzed using SPSS windows version 21.0 software. Results are presented as mean and standard error of mean (SEM), analysis of variant (ANOVA) was used in comparing difference within groups where results were considered significant at $P<0.05$ (95% confidence interval).

RESULTS

Table 1: Showing the effect of carmoisine and acesulfame potassium on body weight.

Body weight (g)					
		MEAN	±SEM	P-VALUE	T-Value
Group A	Initial	135.00	±9.57		
	Final	172.50	±11.08	0.04	-1.861
Group B	Initial	147.50	±7.50		
	Final	157.50	±4.78	0.423	-0.926
Group C	Initial	132.50	±9.46		
	Final	157.50	±8.53	0.206	-1.608
Group D	Initial	152.50	±17.01		
	Final	177.50	±15.47	0.482	-0.801

Result from the table above showed that there was an increase in the body weight of rats in group A, B, C and D at the end of the experimental period (Final weight) when compared to the Initial weight, although only that of the control group A attained statistical significance.

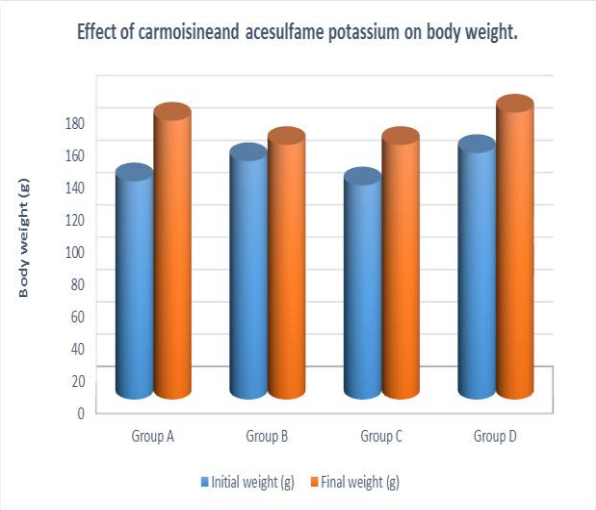

Fig 1: Bar chart showing the effect of carmoisine and acesulfame potassium on body weight of rats.

Table 2: Showing the effect of carmoisine and acesulfame potassium on relative heart weight.

	Mean	±sem	P-value	F-value
Group B	0.43	±0.01	0.018*	
Group C	0.34	±0.01	1.000	5.669
Group D	0.32	±0.00	0.411	

For the relative heart weight, there was a significant increase in group B (0.43 ±0.01), but not in groups C and D compared to the control.

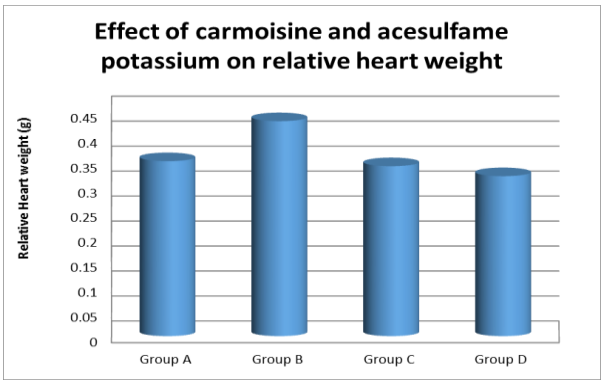

Fig 2: Bar chart showing the effect of carmoisine and acesulfame potassium on relative heart weight.

HISTOLOGICAL FINDINGS

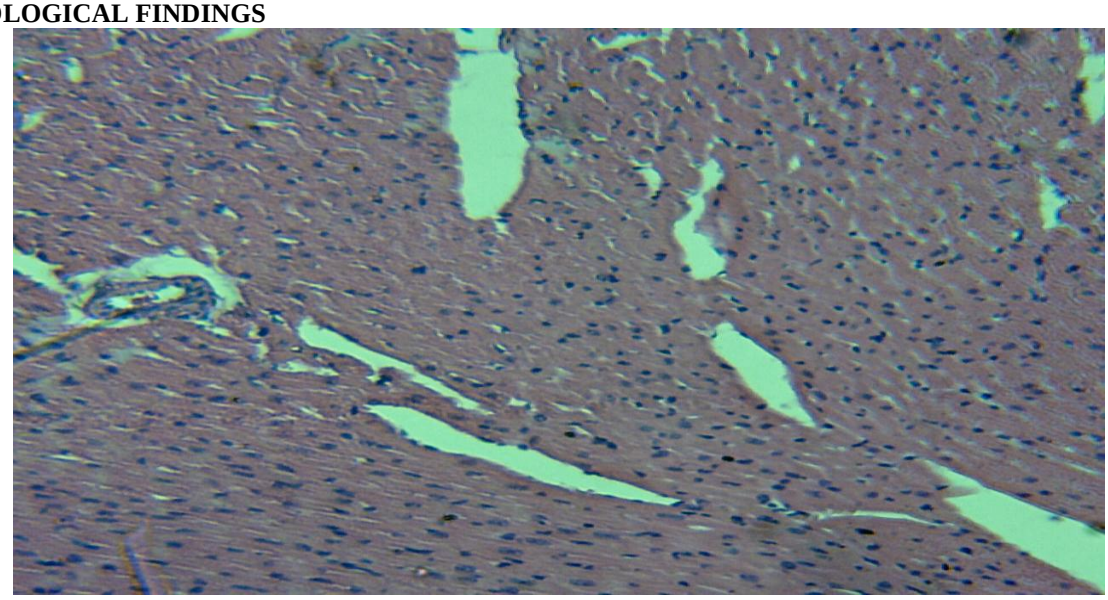

Plate 1: Representative photomicrograph of the heart of Wistar rat from group A (Control); Cardiac muscle, fibres and cells appear normal with no sign of injury (H&E x400).

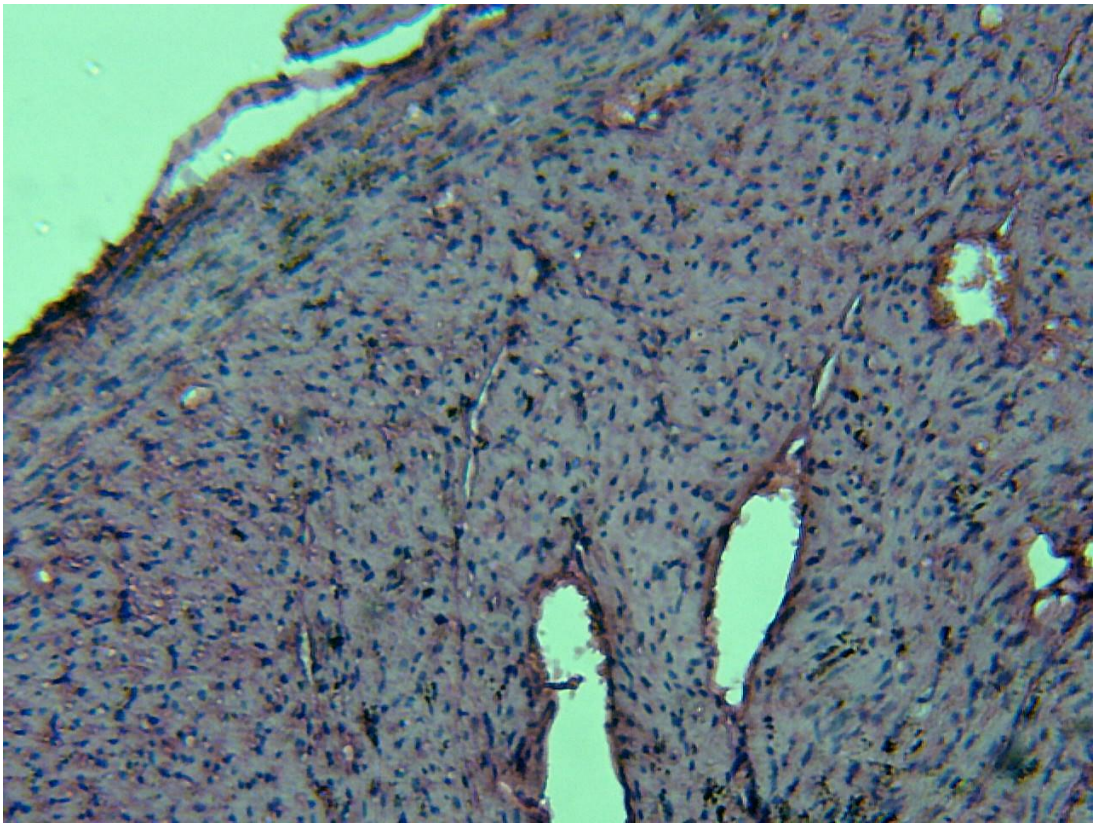

Plate 2: Representative photomicrograph of heart of Wistar rat in group B administered 500mg/bw of carmoisine; Cardiac muscle fibres appears normal but with mild infiltration of inflammatory cells (H&Ex400)

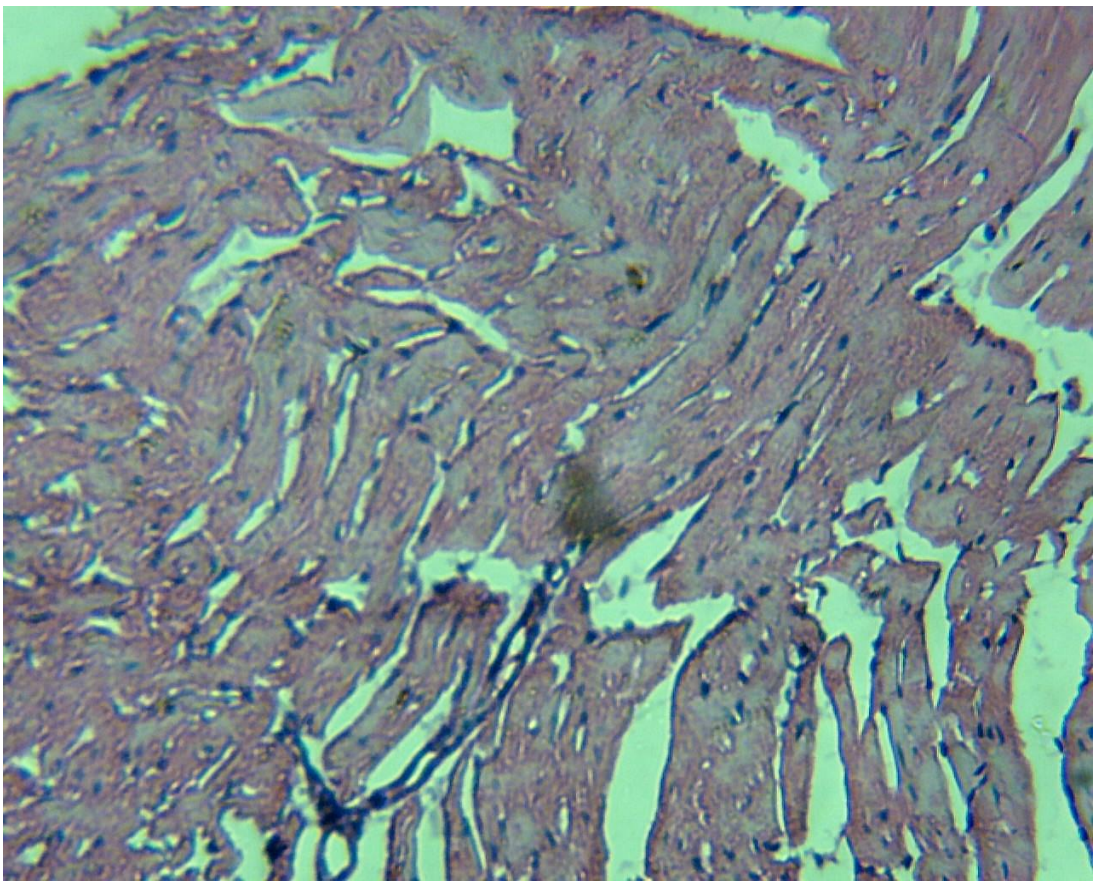

Plate 3: Representative photomicrograph of heart of Wistar rat in group C administered 500mg/bw of acesulfame potassium; Cardiac muscle, fibres and cells appear normal with no sign of injury (H&E x400).

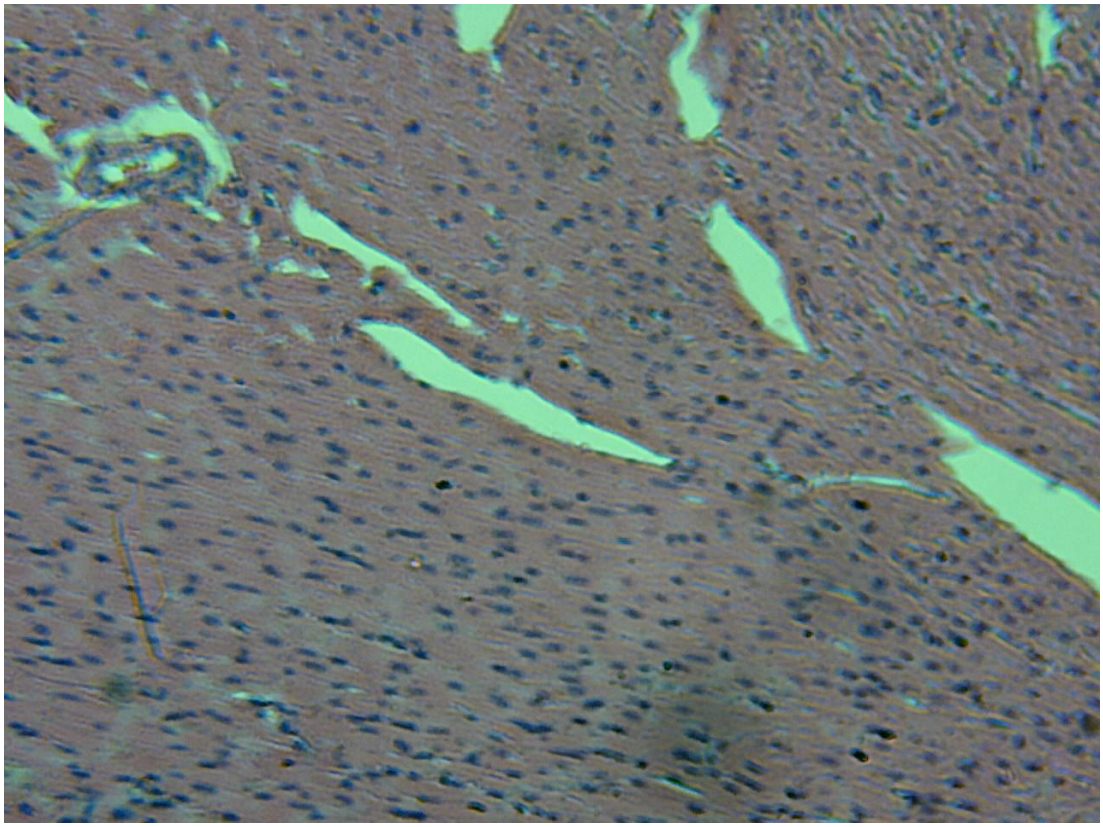

Plate 4: Representative photomicrograph of heart of Wistar rat in group D administered 250mg/kg bw each of carmoisine and acesulfame potassium; Cardiac muscle, fibers and cells appear normal with a few scattered inflammatory cells (H&E x100)

DISCUSSIONS

Observation of body weight showed that there was an increase in body weight but was insignificant in all test groups compared to the control group (Table 4.2) this is similar to the work done by Sharma *et al*., (2005). They studied the hemotoxic effect of chocolate brown on Swiss albino mice and observed no significant change in body weight of experimental mice when compared with control. On the contrary, Sharma *et al*., (2009) showed a highly significant decrease in the body weight of experimental animal when fed with tartrazine; Beenam and Shiv (2014), studied the effect of some food colours on swiss albino rats which revealed a significant increase in body weight. A similar contrary finding was also reported by Gautam *et al.*, (2010), he studied the toxic impact of tartrazine on swiss albino mice and also found an increase in body weight in both experimental groups i.e. for low dose (0.2 g/kg body weight) and high dose (0.4 g/kg body weight) groups. Hasan (2010) also showed significant weight gain in experimental animals treated with 7.5 mg/kg body weight tartrazine and 0.15 and 0.3 mg/kg chocolate brown. Chatterjee and Shinde (2002) also reported an increase in the body weight over 20% above the mean body weight. Similar results have also been reported by Osman *et al*., (1995) in mice fed with synthetic food colorant; Sharma *et al*., (2005) in mice fed with orange red; Sharma *et al*., (2006) in mice fed with apple green and Chakravarty *et al*., (2007) in mice fed with lead chromate. The marked discrepancies observed between the various research studies may be attributed to dose variations as well as the duration of additive intake.

Organ weight is one of the most sensitive drug toxicity indicators, and its changes often precede morphological changes (Piao *et al*., 2013). The significant increase in the relative heart weight of group B which was administered carmoisine only is line with work done by Oyewole and Oladele (2017).

Cardiac muscle fiber of the same group appeared normal with mild infiltration of inflammatory cell (Plate 4). Oyewole and Oladele (2017) from their result, the cardiac tissues were characterized by deformed nuclei, disarray of myofibres and connective tissue deposits which might probably be due to degeneration of the structural protein in mitochondria of the cytoplasm.

CONCLUSION

Results of this study revealed that exposure of rats to carmoisine and acesulfame potassium caused mild distortion of cardiac fibers which may constitute a risk factor for cardiac disorders.

RECOMMENDATION

I recommend that food additives should be avoided as much as possible, if its use become necessary, it should be done with caution and properly monitored by relevant regulatory agencies.

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