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

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ABSTRACT

Carmoisine and acesulfame potassium are widely used as food additives. This study examined the effects of oral administration of carmoisine and acesulfame potassium on some biochemical parameters and histology of the liver of Wistar rats. Twenty adult Wistar rats (average weight 120-200g) were divided into four groups of five animals each. Group A (control) was given rat feed and water, group B received 500mg/kg/bw of carmoisine, group C received 500mg/kg/bw of acesulfame potassium while group D was co-administered 250mg/kg body weight 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 liver tissues were harvested, weighed and fixed in 10% formaline for histological studies. Blood for serum chemistry was collected through ocular puncture for histochemical studies from all the rats. Evaluation of liver function enzymes was carried out using randox kit method. The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), in groups B, C & D were significantly higher ($P<0.05$) than the control group A. Histological observation showed that administration of carmoisine and acesulfame potassium resulted in necrosis of liver tissue and this correlates with the biochemical results. This study revealed that exposure of rats to carmoisine and acesulfame pottassium at these concentrations caused impaired hepatic functions which may constitute a risk factor for hepatic disorders.

KEYWORDS: Carmoisine, Acesulfame Potassium, Liver, Adult Wistar rat.

INTRODUCTION

Food additives (either food dyes or sweeteners) are chemicals added to food with the aim of improving and enhancing their flavour, taste, colour, texture and for preservation(Downham, 2000). In making the food products, many attractive types of natural and synthetic dyes are 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 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). 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 (Pandey and Upadhyay, 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 (Pandey and Upadhyay, 2012). 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. Many consumers may not be aware that these condiments are part of their food. Two main sources of dangerous or threatening additives are those that are put in as part of food processing operations; including the colorings, preservatives, flavours and flavour enhancers, sweeteners, texture agents and processing agents and from food packaging, storing and handling (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 rashes 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 on the other hand is one of the non-nutritive sweeteners that aid 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

This research was carried out in the Department of Anatomy, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. Materials used include; dissecting kit, Electronic weighing balance (Leica CT 250 1101735428, 2012), Absorbent cotton wool, 10% formal saline, Haematoxylin and eosin (H and E) x 100, Dissecting pins, Dash board, 20 adult Wistar rats, wooden 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.

All chemicals used were of analytical grade. 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 acquired from Dubem Chemical store at head bridge market Onitsha.

Experimental Protocol

A total of 20 adult female Wistar rats were obtained from the Department of Physiology, Nnamdi Azikiwe University, Nnewi Campus and was housed in the Central Animal House at Nnewi Campus. The animals weighed from 160-200g. The rats were kept in wooden cages at optimum temperature, 12hrs light/dark cycle and fed with commercial grower smash and water *ad libitum*. Each of the rats were marked for proper identification using permanent markers of different colors. The experiment was carried out in accordance with current rules and guidelines established for care of laboratory animals. The rats were acclimatized for 2 weeks before administration commenced.

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). Administration of food additives was done orally using the oral gavage method. Animals were randomly grouped as follows;

Group A; Served as control that receives normal feed and water daily throughout the period of experiment
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 blood samples were collected by ocular puncture. The liver of each rat was weighed and immediately fixed in 10% formol saline for histological studies using the H&E method.

Statistical Analysis

The result of data (body weight changes and liver function tests) was analyzed using SPSS version 21.0. Results are presented as Mean ± Standard error of mean (SEM), analysis of variance (ANOVA) was used in comparing difference within groups and results were considered significant at $P<0.05$.

RESULTS

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

Body weight (g)		MEAN	±SEM	P-VALUE	T-Value
Group A	Initial	135.00	±9.57		
	Final	172.50	±11.08	0.160	-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 no significant increase in the body weight in group A, B, C and D at the FINAL when comparing to the INITIAL weight.

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

GROUP	MEAN	±SEM	P-VALUE	F-VALUE
A	0.27	±0.06		
B	0.31	±0.04	0.001*	
C	0.34	±0.05	0.000*	30.377
D	0.30	±0.03	0.010*	

The result in Table 2 above shows higher liver weight in the experimental groups B, C and D compared to that of group A.

Table 3: Showing the effect of carmoisine and acesulfame potassium on Liver Enzymes aspartate amino transaminase, alanine transaminase and alkaline phosphatase.

ENZYME GROUP		MEAN	±SEM	P-VALUE	F-VALUE
AST (U/L)	A	7.67	±0.33		
	B	17.00	±0.57	0.000*	
	C	18.66	±0.33	0.000*	140.667
	D	14.67	±0.33	0.000*	
ALT (U/L)	A	9.67	±0.33		
	B	14.67	±1.45	0.003*	
	C	16.00	±0.57	0.001*	11.153
	D	13.33	±0.33	0.013*	
ALP (U/L)	Group A	42.00	±11.93		
	Group B	87.66	±13.01	0.015*	
	Group C	95.33	±10.26	0.007*	5.236
	Group D	85.00	±5.03	0.020*	

The results of the liver function tests as presented in Table 3 shows significant increases in serum levels of ALT, AST and ALP compared to the control group.

Histological findings

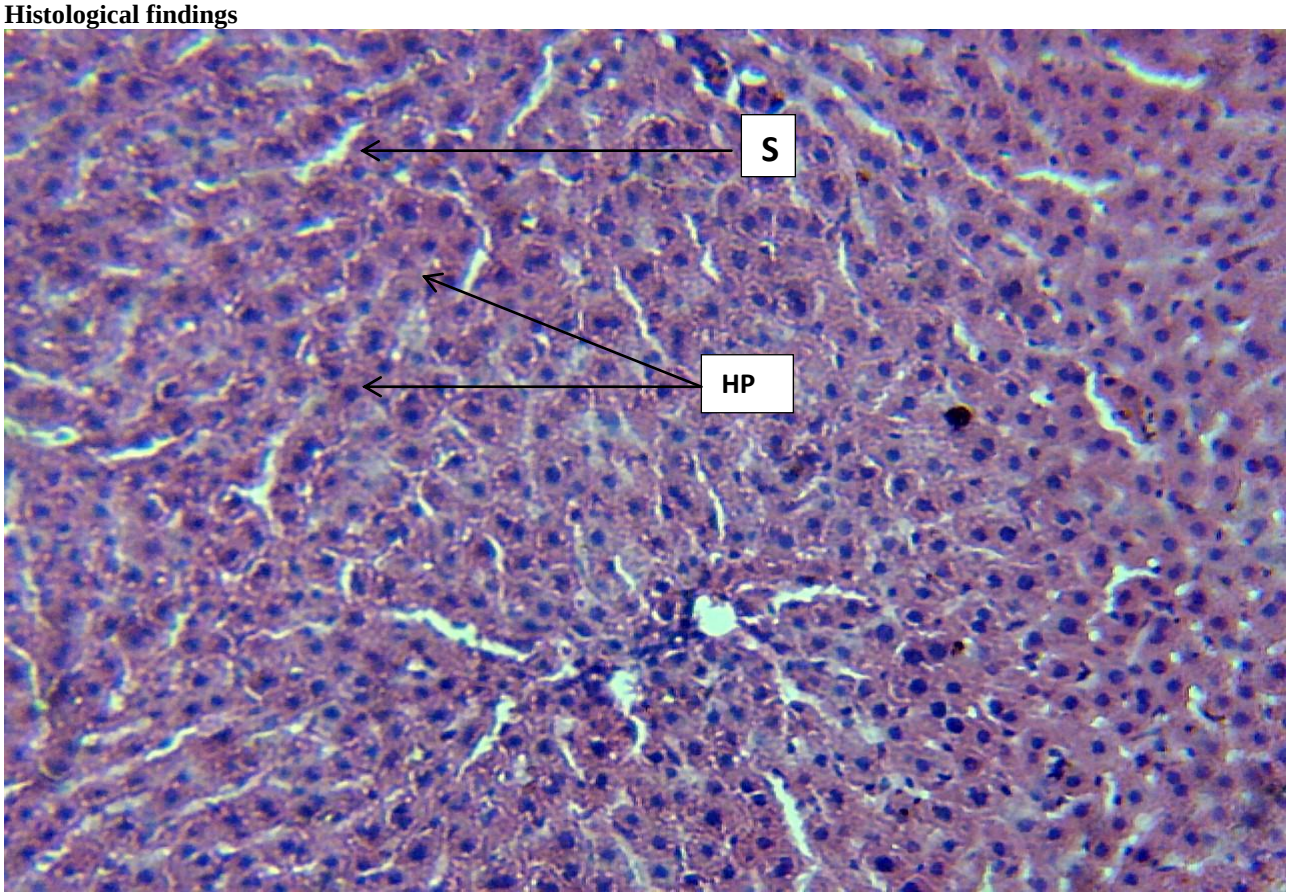

Plate 1: The photomicrograph of liver of female Wistar rat from the control group showing normal histology. Hepatocytes (HP) and sinusoids (S) are intact (H&E X 100).

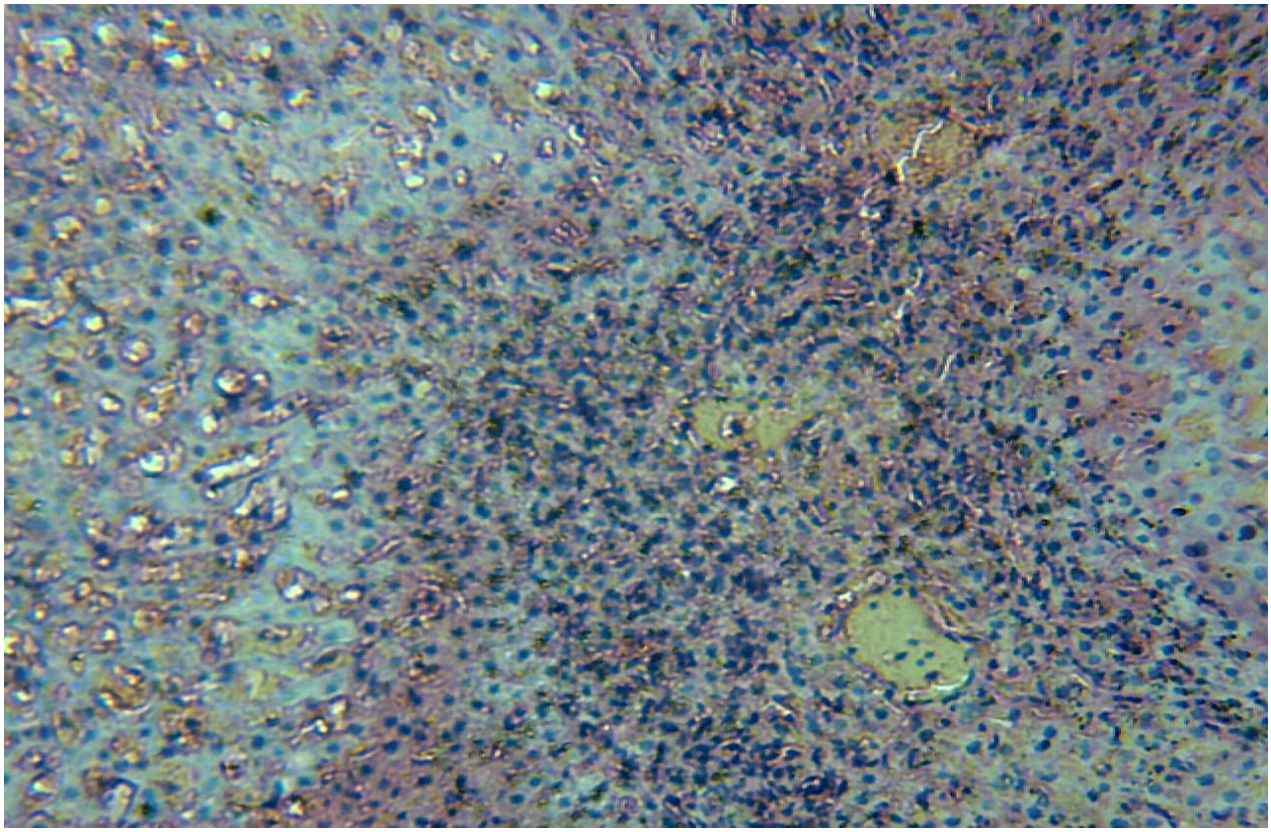

Plate 2: The photomicrograph of liver of female Wistar rat in Group B administered 500mg/bw of carmoisine; shows gross distortion of liver architecture with profuse signs of tissue necrosis.

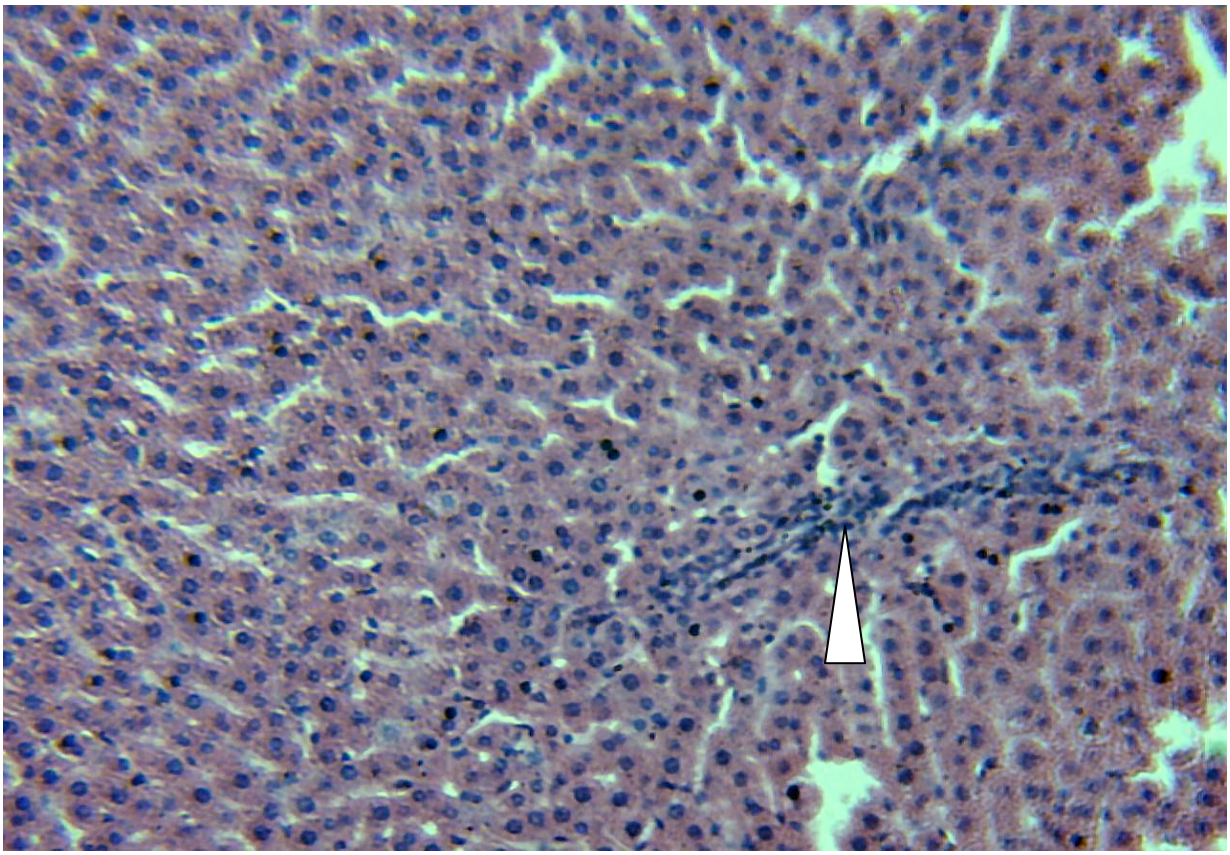

Plate 3: Photomicrograph of liver of female Wistar rat administered 500mg/bw of acesulfame potassium; liver shows mild infiltration of inflammatory cells and some extent of tissue necrosis (H&E x100).

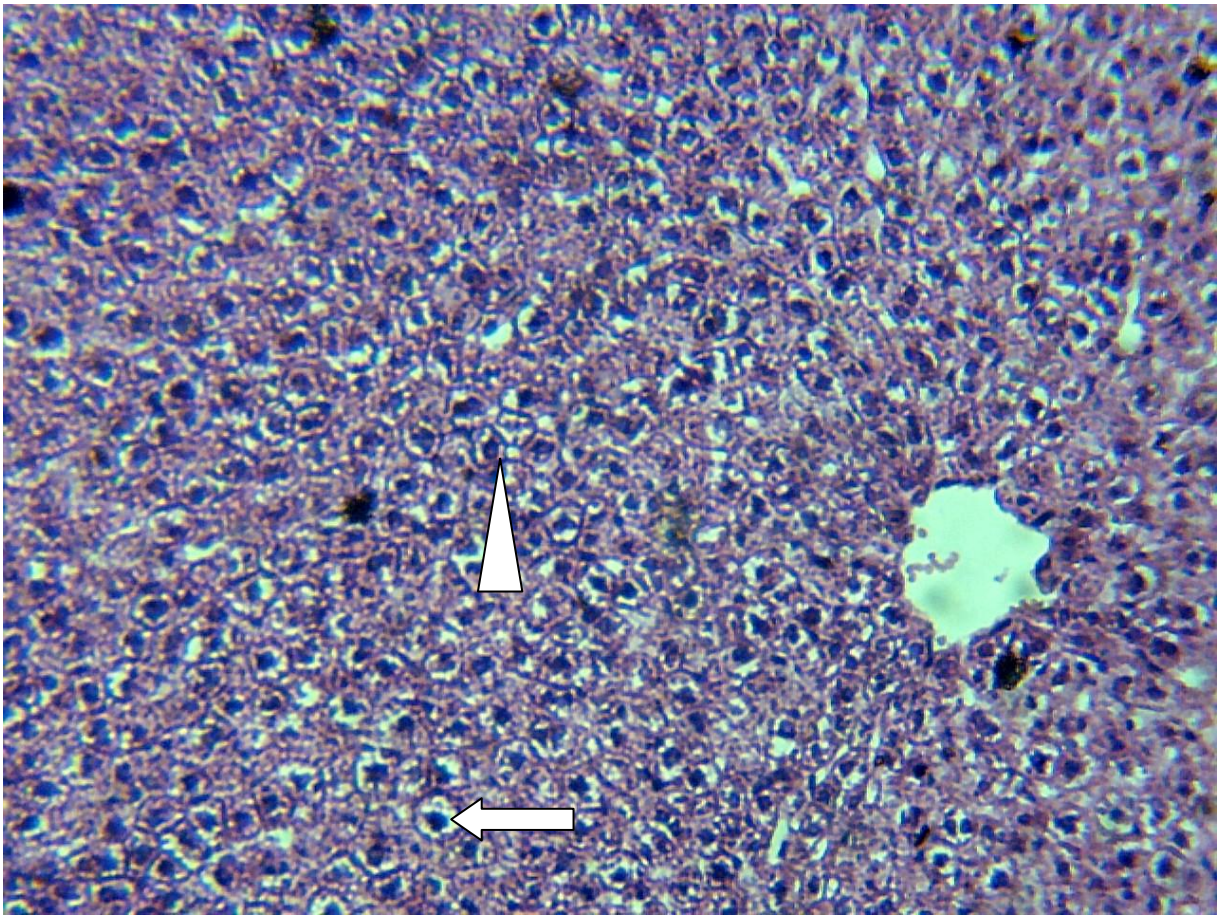

Plate 4a: The photomicrograph of liver of female Wistar rat co-administered 500mg/bw of carmoisine and acesulfame potassium each; shows high degree of cellular dysplasia. There is proliferation of hyper chromatic atypical hepatic cells (arrow head), displaying high degree of peri nuclear hollow (arrow head) (H&E x 100).

DISCUSSIONS

Food additives are widely used in factory-made foods. Therefore, they must be completely safe for human consumption. Nevertheless, scientific studies on these additives have yielded unfavorable results (Downham 2000).

Physical observation from this study revealed that the intake of carmoisine only caused some behavioral changes in adult Wistar rats; hyper activity was observed in this group compared to the control group and other test groups.

Result of body weight showed that there was no increase in body weight in all the rats (Table 1). This is contrary to the report of Sharma *et al*., (2009) which showed a highly significant decrease in the body weight of experimental animal when fed with tartrazine. Beenam and Shiv (2014) also studied the effect of some food colours on swiss albino rats and reported 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 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*., (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 (Ying et al., 2013). The relative organ weight result also showed significant differences in groups. There was significant ($P>0.05$) increase in the relative liver weight in group B, C and D (table 2) with these values; group B (3.10±0.04), C (3.43±0.05) and D (3.00±0.03) when compared to group A (2.76±0.06).

The measurement of the activities of marker enzymes in tissues and body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organ/tissues (Malomo, 2000; Yakubu,

2003). Measurement of enzyme activities can also be used to indicate tissue cellular damage caused by a chemical compound long before histological changes (Akanji, 1986).

Alkaline phosphate is a membrane bound enzyme (Wright and Plummer, 1974), while aspartate amino transaminase and alanine transaminase are cystolic enzymes (Schmidt et al., 1965). These enzymes are highly concentrated in the liver and kidney and are only found in serum in significant quantities when the cell membrane becomes leaky and even completely ruptured (Cotran et al., 2005). A rise in serum level or decrease in tissue level of these intracellular enzymes is an index of damage to the liver and kidney cells (Moss and Rosalki, 1996).

The serum levels of group B (administered carmoisine only) showed a significant increase in aspartate amino transaminase level (7.67±0.33), alanine aminotransaminase and alkaline phosphate when compared with group A.

For group C (administered acesulfame potassium only), there was also a significant increase in aspartate amino transaminase level (18.66±0.33, Alanine aminotransaminase and Alkaline Phosphatase when compared with group A.

In group D, there was also a significant increase in the activities of enzymes; aspartate amino transaminase, alanine transaminase, alkaline phosphate; (14.67±0.33), (13.33±0.33) and (85.00±5.03) respectively.

The significant increase in these enzymes is in line with the work done by Imafidon *et al.*, (2016). Ford *et al*., (1987) and Brozelleca and Hallagan (1988a, b) stated that carmoisine and tartrazine caused insignificant changes in rat serum AST, ALT and ALP.

The observed increase in the activities of enzymes in the liver is suggestive of liver failure. Enzyme activities in tissues are often used as marker to ascertain early toxic effects of administered foreign compounds to experimental animals (Adesokan and Akanji, 2004). Work done by Amin *et al*., (2010) evaluated the toxic effect of carmoisine on hepatic function, lipid profile, blood glucose, body-weight gain and biomarkers of oxidative stress in tissue. They reported that carmoisine affects the liver tissue adversely and alters biochemical markers in liver. Increased level of ALT often suggests the existence of other medical problem such as viral hepatitis, diabetes, congestive heart failure liver damage, infectious mononucleosis or myopathy (Paul and Giboney, 2005). An increase in ALT may also be caused by dietary choline deficiency. AST is widely distributed in animal tissues, being more concentrated in the liver, heart and skeletal muscle. As the concentration of AST is very high in the heart, large amount of the enzyme is released into the circulation in myocardial infarction.

The administration of carmoisine only (group B) caused a severe gross distortion of liver architecture with profuse sign of tissue necrosis. Damage to liver is evidenced by a significant increase in aspartate amino transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Work done by Aboel-Zahab *et al*., (1997) supports this claim. Similar work carried out by Xiaoguang *et al.,* (2014) who conducted their study on damaging effect of food additive showed a series of histopathologic changes in mouse livers, varing degrees of necrotic changes in the liver, severe injury to liver structure, hepatocyte necrosis small venule bleeding, inflammatory infiltration around the central vein.

CONCLUSION

Results of this study revealed that exposure of rats to carmoisine and acesulfame potassium adversely affected liver histoarchitecture and led to disturbing increases in serum liver function enzyme. This may constitute a risk factor for general liver function.

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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