FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 3, June, (Special Issue) 2024, pp 228 - 232
DOI: https://doi.org/10.33003/fjs-2024-0803-2434

TOXICOLOGICAL STUDY AND QUANTITATIVE DETERMINATION OF ALLURA RED (E129) DYE ADDITIVE IN SOME FOOD BEVERAGES CONSUMED IN KATSINA METROPOLIS, NIGERIA

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ABSTRACT

The continuous use of synthetic dyes in beverage foods as additives for consumers' attraction could lead to many health-related issues that include cancer, allergic reactions etc. These have leads to urgent monitoring of the amount of such colorants in foods and beverages. Experimentally, the acute toxicity (LD_{50}) was carried out on the Allura red (E129) dye using wistar albino rats in accordance with the administered doses per body weight of the tested animals, as well as quantitative determination of the dye analyte in some food beverages consumed in Katsina metropolis, Nigeria. However, the quantitatively analyzed samples were found to contain 115.294 ± 0.013, 109.142 ± 0.115, 117.173 ± 0.037, 118.385 ± 0.080 and 123.203 ± 0.051 mg/L of the Allura red (E129) dye in sample A, B, C, D and E, respectively. Also, the LD_{50} result of the Allura red (E129) dye standard was estimated to be more than 5000 mg/L per body weight of the tested animals. Even though, there are few changes in the animals' behavioral attitudes, which vary according to the concentration of doses administered and the results produced no mortality at the given doses range of 50 to 5000 mg/L after administering the dye standards. Therefore, the results justify the unsafe consumption of the analyzed beverages since the concentration of the Allura red (E129) azo dye in them is above the maximum permissible limits of 50 mg/L as supported by the Guideline for the Testing of Chemicals, Acute Oral Toxicity–Acute Toxic (OECD, 2001).

Keywords: Allura dye, Concentration in Food Beverages, LD_{50}, Wistar albino rats, UV-Visible Spectrophotometer

INTRODUCTION

Belitz *et al.* (2009) defined food additives as substances that are added to food during production, processing, packaging and storage of food. Moreover, additives are added to food to increase or improve values that include nutrients, odor, texture, taste and serve as preservatives to protect against microbial attacks as well as a color attraction (Pașca *et al.*, 2018). For instance, synthetic azo dyes are the most widely used additives in modern days due to their high color attraction that eventually adds to economic value in products such as foods, pharmaceuticals and beverages by small and large scale industries (Dey & Nagababu, 2022; Liu *et al.*, 2011). Unfortunately, synthetic dyes and other forms of contaminants that include pesticides (Abdulra'uf & Lawal, 2020; Abdulra'uf *et al.*, 2019; Lawal & Abdulra'uf, 2021, 2022; Lawal & Abdulra'uf, 2020; Lawal *et al.*, 2020; Lawal & Haliru, 2021; Lawal & Ibrahim, 2023; Lawal & Koki, 2019; Lawal & Low, 2021; Yarima *et al.*, 2021), polycyclic aromatic hydrocarbons (PAHs) (Chander, 2014), mycotoxins (Alsharif *et al.*, 2016; Alsharif *et al.*, 2015), heavy metals (Doro *et al.*, 2021a, 2021b; Gafai *et al.*, 2023; Gafai *et al.*, 2024; Koki *et al.*, 2018; Lawal & Shafi'u, 2020), pharmaceutical residues (Aliu *et al.*, 2023; Junaid *et al.*, 2023; Lawal & Muhammad, 2022; Sani & Lawal, 2024) etc. However, these contaminants have been reported to be toxic after a long time of accumulation in the body tissues, which results in health-related issues that include different forms of cancers etc (Bafana *et al.*, 2011). Consequently, there is a need for monitoring the amount of such kind of colorants in the daily consumed foods and beverages since there are limited reports in many areas.

The objective of this study is to carry out Acute toxicity (LD_{50}) study of the Allura Red (E129) azo dye (Fig. 1) in wistar albino rats using the method revealed by OECD (2001). And to determine the concentration levels of Disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl) azo]-2-naphthalenesulfonate [Allura Red (E129) azo dye] in selected beverages by adopting the reported method (Antakli *et al.*, 2015). Therefore, it is hoped that this study will create awareness and guide against excessive usage of such products in foods and beverages by many consumers and could also serve as a reference guide for future studies.

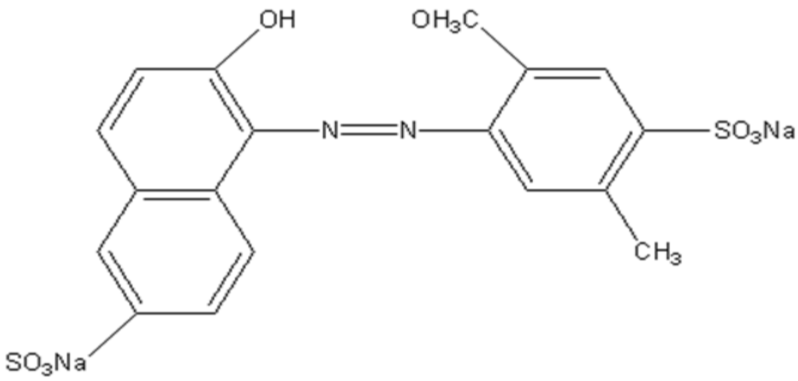

Figure 1: Structural formula of Allura red dye

MATERIALS AND METHODS

Sampling and Sample Preparation

The Tianjin Kemiou Chemical Reagent Co. Ltd, China supplied the standard dye of an analytical grade. The following materials containing the Allura red dye; Foster Clerks/berries (Sample A), Fearless/red berry (Sample B), Bevi-Mix/berries (Sample C), Fresh Quick/berries (Sample D), and Tiara strawberry (Sample E) were sourced in Katsina central market, Nigeria.

Preliminarily, the stock solution of 100 mg/L of the dye standard was prepared by dissolving 0.01 g of the dye was transferred into 100 mL volumetric flasks. Then, 20 mL of methanol was added to dissolve the dye before mixing it with distilled water and filling it to the mark. The 5, 10, 15, 20, 25, and 30 mg/L working standard solutions of the dye were prepared using the dilution formula (equation 1), respectively.

$$C_1V_1 = C_2V_2 \quad (1)$$

Where,

C_1 = Initial concentration of undiluted solution,

V_1 = Initial volume of undiluted solution,

C_2 = Final concentration of diluted solution,

V_2 = Final volume of diluted solution.

Moreover, the prepared standard solutions were used for the calibration of the UV-visible spectrophotometry instrument using 497 nm wavelengths. Fortunately, the regression coefficient of linearity (R^2) is close to one (0.999). The linear equation (equation 2) of the graph's calibration curve was acquired from the Excel Microsoft software as graphically illustrated (Fig. 2). Thus, the linear equation was used for estimating the concentrations of the targeted dye analytes in the analyzed samples.

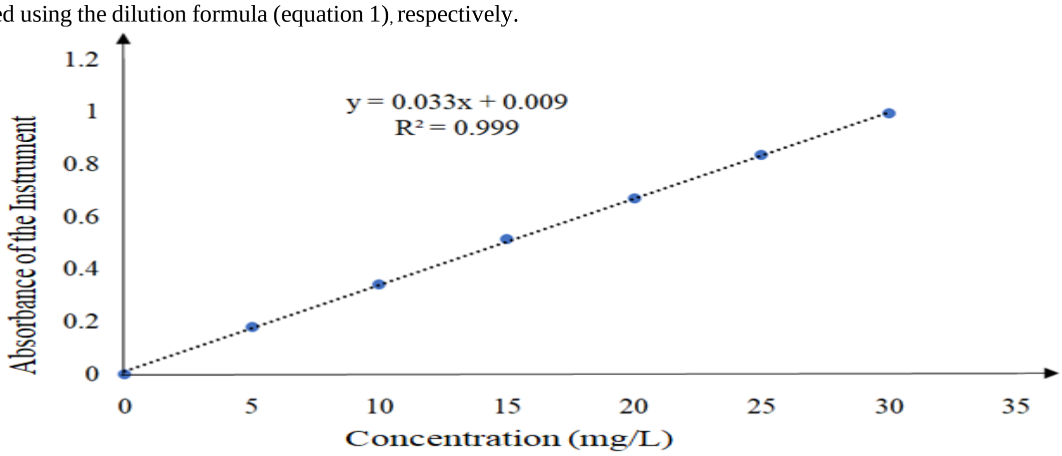

Figure 2: The calibration curve of the instrument

$$y = 0.033x + 0.009 \quad (2)$$

Where,

y = Absorbance or peak area reading of the instrument,

x = Concentration

Methodologically, the five (5) brand samples (A - E) for each of the beverages and soft drinks were purchased from the traders of Katsina Central market, Katsina State (Nigeria). The 1 g of the powdered sample was dissolved in a 25 mL volumetric flask and filled to the mark with distilled water. The solution was transferred into a centrifuge-tube and centrifuged for 15 min at 5000 rpm. The obtained analyte solution was preserved at 4 ^oC in a screwed bottle before the UV-visible Spectrophotometric analysis. Similarly, the liquid samples such as soft drinks were degassed in a water bath at 25 ^oC for 15 min and preserved at 4 ^oC in a screwed bottle as well before the analysis as revealed by Antakli *et al*. (2015). Then, the analyte solutions were analyzed with a UV-visible spectrophotometry instrument and the absorbance readings were recorded. Finally, the concentration (mg/L) of Allura red (E129) azo dye in the selected beverages of the targeted analyte was estimated and recorded.

LD_{50} (Acute) Toxicity Study

In this study, the twelve (12) Wista albino rats with an average weight of 420 g were purchased from the Animal Farm of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria. The rats were grouped into 4 with respect to their close-ranged body weights, where each group is made up of 3 rats per cage and named Group O, A, B, and C. The test animals were fasted overnight by only providing them with water, after which the dye solutions were administered orally to each of the rats at the dose levels of 0, 50, 500 and 5000 mg/L body weight of the rats, respectively. Resultantly, the mortality and changes in behavioral attitudes of the test animals after administration of dosages were monitored from the beginning through till the 72 hours period of observations. The observations include behavioral attitudes of the test animals and mortality of the tested animals were recorded in accordance with the administered doses (OECD, 2001).

RESULTS AND DISCUSSION

The concentration results of Allura red (E129) dye obtained in mean ± standard deviation (π ± SD) of the analyzed samples are shown in Table 1 and figuratively expressed in Fig. 3, respectively.

Table 1: Concentration values of Allura red (E129) dye present in the analyzed samples

Sample Code	Sample Absorbance	Concentration (π ± SD) Obtained (mg/L)
Sample A	3.814	115.294 ± 0.013
Sample B	3.611	109.142 ± 0.115
Sample C	3.876	117.173 ± 0.037
Sample D	3.916	118.385 ± 0.080
Sample E	4.075	123.203 ± 0.051

Key: Sample A, Foster Clerks (berries); Sample B, Fearless (red berry); Sample C, Bevi Mix (berries); Sample D, Fresh Quick (berries); Sample E, Tiara (strawberry)

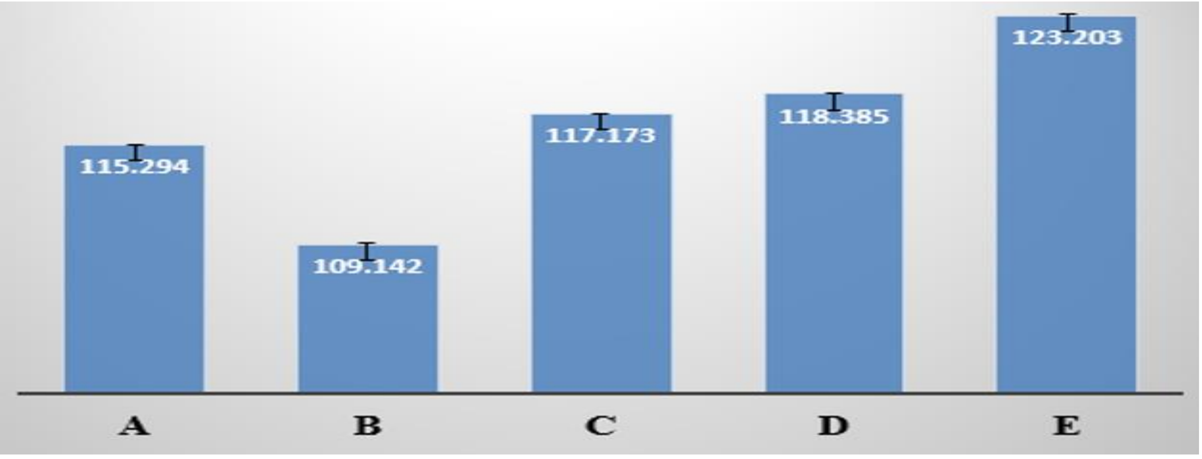

Figure 3: The concentration (mg/L) of Allura red (129) dye in the analyzed samples

The concentration of Allura red (E129) dye obtained in samples A (115.294 ± 0.013 mg/L), B (109.142 ± 0.115 mg/L), C (117.173 ± 0.037 mg/L), D (118.385 ± 0.080) and E (123.203 ± 0.051) were higher than 87.887 mg/L in a confectionery sample of Ibon fruity candy as reported by Lawal *et al.* (2021). Unfortunately, all the concentrations were higher than the maximum permissible limits (MPL) of 50 mg/L of Allura red (E129) dye for the non-alcoholic flavored drinks, dried fruits and vegetables as documented by CAC (2008).

In addition, Table 2 shows the results of the acute toxicity (LD_{50}) study of Allura red (E129) azo dye using Wista albino rats in accordance with the administered doses per body weight of the tested animals, as well as their behavioral attitudes and mortality.

Table 2: Result of the acute toxicity study of Allura red (E129) dye

Group	Dose (mg/L)	Mortality	Change in Behavioral Attitude						
			Salivation	Itching	Coloration	Freedom	Apatite	Lack of Anxiety	Movement towards corners
O	0	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
A	50	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)
B	500	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(+)
C	5000	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(+)

Key: (+), present; (-), absent

However, the result of the LD_{50} values for the Allura red (E129) dye was estimated to be more than 5000 mg/L per body weight of the tested animals. This implies that 50 % or half of the group of tested animals can survive any dose concentration between 1 – 5000 mg/L per body weight of the tested animals but may not survive any concentration above that (OECD, 2001). Thus, the results produced no mortality at the given doses range of 50 to 5000 mg/L after administering the dye standards per body weight of the tested animals but there were changes in their behavioral attitudes, which varies according to the concentration of doses administered. Thus, the result agrees with the report of Abad-Fuentes *et al.* (2015) who show that most of the azo dyes used as food additives and in textiles have very low oral acute (LD_{50}) toxicity as measured in various animals such as rats and rabbits (below 10,000 mg/L), and dogs (5000 mg/L) per their respective body weights.

CONCLUSION

The quantitative assessments of Allura red (E129) dye in some beverages, which are mostly consumed within the metropolitan parts of Katsina (Nigeria) as well as the oral

LD_{50} (acute toxicity) were successfully carried out. Therefore, the results justify the unsafe consumption of the analyzed beverages since the concentration of the Allura red (E129) azo dye in them is above the maximum permissible limits of 50 mg/L as supported by OECD, 2001 (Guideline for the Testing of Chemicals, Acute Oral Toxicity–Acute Toxic). The excessive consumption of such beverages containing the dye additives could lead to continuous accumulation of the dye in the body tissues far beyond the maximum permissible limits of the dye, which may result in health issues in the long run.

ACKNOWLEDGEMENT
The TETFund Research Grant is acknowledged for the support rendered.

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