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Quantitative Assessment and Toxicological Study of Sunset Yellow Dye (E110) as an Additive in some Beverages Consumed in Katsina Metropolis, Nigeria

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ABSTRACT

Synthetic dyes have been used in food, beverages and pharmaceuticals for consumer attractions. The dyes are usually added to substrates to replace the natural colorants that could be lost during processing or to prevent variations in the color of the final products. Unfortunately, the dyes have been reported to cause many health-related issues. However, there is a need to constantly monitor the amount of such colorants in our food and beverages. Empirically, the acute toxicity was carried out to examine the LD_{50} (acute toxicity) of the Sunset yellow (E110) (analyte) dye using Wistar albino rats in accordance to the administered doses per body weight of the tested animals, as well as quantitative analysis of the targeted analyte in some beverages consumed in Katsina metropolis, Nigeria. The result of the LD_{50} values for the Sunset yellow dye standards estimated to be more than 5000 ppm per body weight of the tested animals. Thus, there were some few changes in the animals' behavioral attitudes, which varies according to the concentration of doses administered and the results produced no mortality at the given doses range of 50 to 5000 ppm after administering the dye standards. The quantitatively analyzed samples contained 49.536 ± 0.004, 109.785 ± 0.130, 108.975 ± 0.075, 46.140 ± 0.018 and 42.059 ± 0.009 ppm of the Sunset yellow dye, respectively in samples A, B, C, D and E. This justifies the safe consumption of sample A, D and E of the analyzed beverages since the concentrations of the Sunset yellow dye in them were below the maximum permissible limits of 50 ppm as supported by OECD (Guideline for the Testing of Chemicals, Acute Oral Toxicity–Acute Toxic). Although, excessive consumption of such beverages containing the dye additives could lead to continuous accumulation of the dye in the body tissues beyond its maximum permissible limits, that may result in health issues at long run that include different forms of cancers and also provoking allergic reactions such as asthmatic symptoms in the body.

Keywords: Beverages, Sunset Yellow dye (E110), Toxicological studies, Wistar albino rats

INTRODUCTION

Food additives are substances (or a mixture of substances), which are added to food or they may be involved in the production, processing, packaging and storage of food without being a major ingredient (Belitz *et al.*, 2009). There are several purposes or benefits of adding some materials to food items, which serves as additives in food. These benefits include increasing the nutritive value of food; sensory value such as color, odor, taste and texture, as well as serving as preservatives to extend shelf life of foods by protection against microbial spoilage (Pașca *et al.*, 2018). For instance, dye is a colored substance that bonds chemically to the substrate, to which it is being applied (Gürses *et al.*, 2016). For that matter, 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, the synthetic dyes (Lawal *et al.*, 2020) and the other forms of contaminants that include pesticides (Abdulra'uf & Lawal, 2020; Abdulra'uf *et al.*, 2019; Lawal & Abdulra'uf, 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.*, 2021; Gafai *et al.*, 2024; Koki *et al.*, 2018), pharmaceutical residues (Aliu *et al.*, 2023;

Junaid *et al.*, 2023) etc. However, these contaminants have been reported to be toxic after a long-time accumulation in the body tissues, which results in health-related issues that include different forms of cancers etc (Bafana *et al.*, 2011).

Although, the health-related issues caused by the azo dyes is due to the chemical properties of the highly described colored organic compounds with the functional group R−N=N−R′, in which R and R′ are usually aryl that are broken down in the body after consumption into aromatic amine or arylamine (Alabdraba & Bayati, 2014). This process occurs either through reductive cleavage that can be catalyzed by enzyme at a suitable temperature (Mahmood *et al.*, 2016). And a compound such as benzidine is among the cleaved products obtained from the degradation of azo dyes, which were reported to cause cancer in human while others are biochemical makers provoking allergic reactions that include asthmatic symptoms (Monisha *et al.*, 2023). Consequently, there is need to constantly monitor the amount of such kind of colorants in the daily consumed foods and beverages since there are limited reports in many area.

The aim of this study is to determine the acute toxicity (LD50) level by measuring concentration levels of disodium 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonate (Sunset yellow azo dye) in selected beverages via standard methods (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.

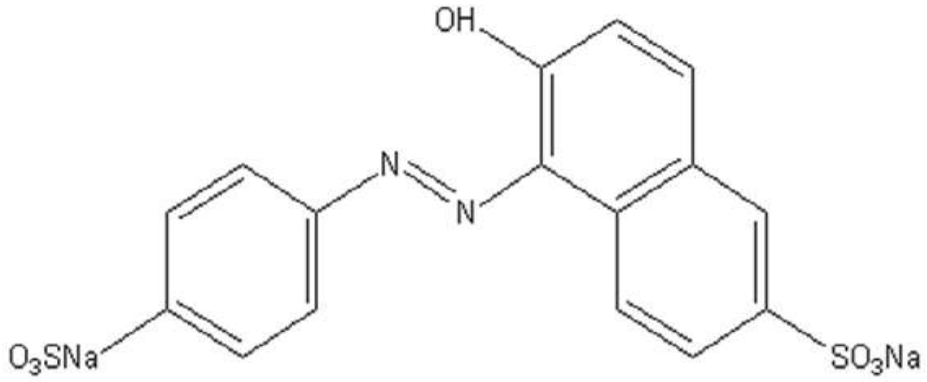

Fig. 1: Structural formula of Sunset yellow dye

MATERIALS AND METHODS

Sampling and Sample Preparation

The analytical grade standard dye was purchased from Tianjin Kemiou Chemical Reagent Co. Ltd, China.

The stock solution of the dye (100 ppm) was prepared by dissolving 0.01g of the standard dye into 100 mL volumetric flasks. Distilled water was added and stirred well to get homogenized before filling it up to the mark. The working standard solutions (5, 10, 15, 20, 25, and 30 ppm) of the dye were prepared using the dilution formula $C_1V_1 = C_2V_2$, respectively. The prepared standard solutions were used for the calibration of the UV-visible spectrophotometer at the wavelength of 419 nm. The linear equation (equation 1) acquired from calibration curve of the graph was used for estimating concentrations of the targeted dye (analytes) in the analyzed samples.

$$y = 0.032x + 0.001 \quad (1)$$

Where, y: Absorbance reading of the instrument,
x: Concentration

Five (5) brand samples each for beverages and soft drinks were sampled (purchased) from the traders of Katsina central market, Katsina State (Nigeria). The analyte (extract) solution was prepared by dissolving 1 g each of the powdered sample of the beverages into 25 mL volumetric flask and fill to the mark with distilled water. The solution was transferred into a centrifuge-tube and centrifuged (5000 rpm) for 15 min. The obtained supernatant extract (analyte solution) was preserved (4 ^oC) in a screwed bottle prior to the UV-visible Spectrophotometric analysis. Similarly, the liquid samples such as soft drinks were degassed in a water bath at room temperature (25 ^oC) for 15 min and preserved (4 ^oC) in a screwed bottle as well before the analysis (Antakli *et al.*, 2015). Then, the analyte solutions were analyzed with UV-visible spectrophotometer and the absorbance readings were recorded. Finally, the concentrations (ppm) of Sunset yellow azo dye in the selected beverages of the targeted analyte was estimated and recorded.

Acute Toxicity (LD_{50}) Study

In this study, the twelve (12) Wistar albino rats with 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 four groups (with 3 rats per group) and the grouping was done based on 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 dyes solutions of 50, 500 and 5000 ppm were prepared by dissolving 0.021, 0.210 and 2.10 g of the standard dye powder respectively into 2 mL syringe. Then, each of the syringe was filled with distilled water to the mark and agitated vigorously for the solutions dissolved. The solutions were administered orally to each of the rats at the dose level of 50, 500 and 5000 ppm per body weight of the rat, respectively. While the Group O rats were only administered with the 2 mL of distilled water. Resultantly, the mortality and changes in behavioral attitudes of the tests animals after administration of dosages were monitored from the beginning through till 72 hours period of observations. The observations made including 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

From the results, the mean concentrations of Sunset Yellow dye obtained ($\pi \pm SD$) of the analyzed samples is shown in Table 1 and figuratively expressed in Fig. 2, respectively.

Table 1: Concentration of Sunset yellow dye in the analyzed samples

Sample Code	Sample Absorbance	Mean Concentration (ppm)
Sample A	1.591	49.536 ± 0.004
Sample B	3.525	109.785 ± 0.130
Sample C	3.499	108.975 ± 0.075
Sample D	1.482	46.140 ± 0.018
Sample E	1.351	42.059 ± 0.009

Key: Sample A, Tiara (Strawberry); Sample B, tiara (mango); Sample C, Jolly Cola; Sample D, Euro (Pineapple); Sample E, Fanta (Orange)

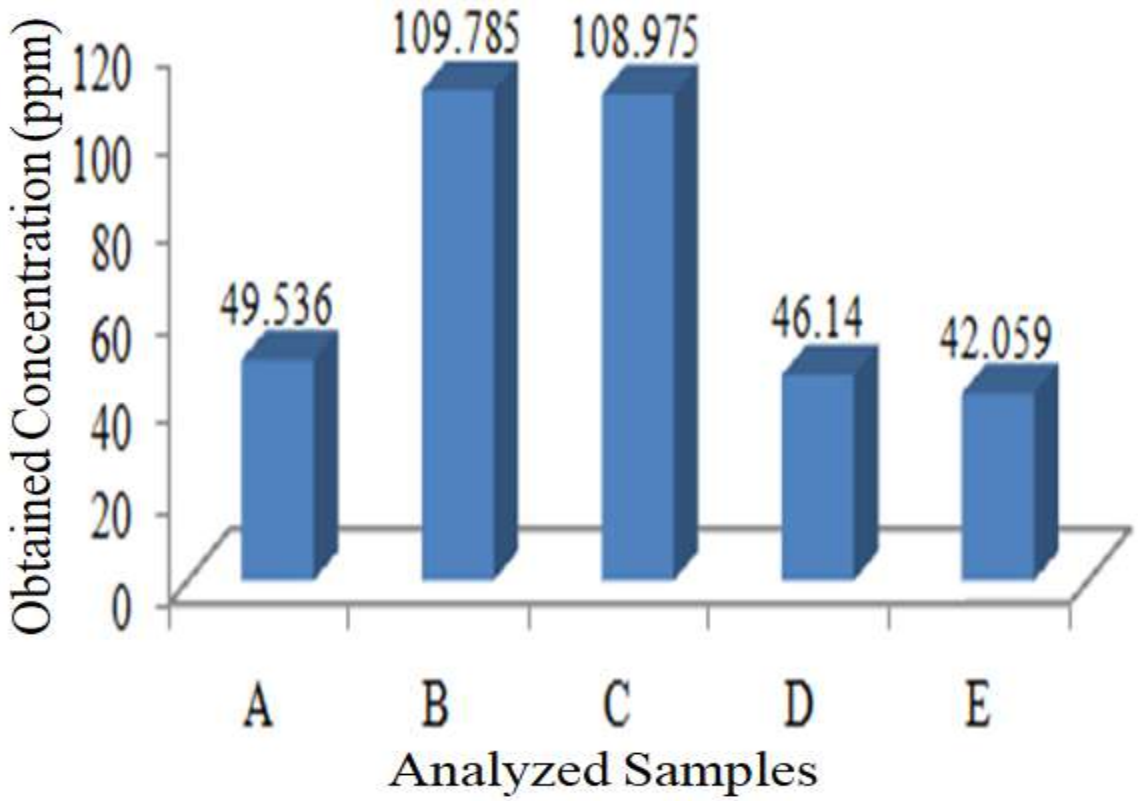

Fig. 2: The concentrations of Sunset yellow dye obtained in the analyzed samples

The concentrations of Sunset Yellow dye obtained in sample B (109.785 ±0.130 ppm) and C (108.975 ± 0.075 ppm) were higher than 87.887 ppm in a confectionery sample of Ibon fruity candy (Lawal *et al.*, 2021) and also found higher than the maximum permissible limits (MPL) of 50 ppm of Sunset yellow dye for the non-alcoholic flavored drinks, dried fruits and vegetables as documented by CAC (2008). Meanwhile, samples A, D and E have lower concentrations of the dye; 49.536 ± 0.004, 46.140 ± 0.018 and 42.059 ± 0.009 ppm, respectively and were found to be in the same

range with the values that were recently documented (Lawal *et* al., 2021); 46.523, 49.364, 41.924, 27.853, 52.311, 33.045, 29.515, 34.250 and 33.295 ppm in confectionery samples of I Love fruits candy, Crash tropical flavors, Cimon fruity peach, Crush tancy orange, Toffix multi-vitamin, Love pop lollipop, Love pito lollipop, Orange lollipop and Mentos orange. This was fortunately found below the MPL of 50 ppm for Sunset yellow dye (CAC, 2008).

Moreover, Table 2 showed the results of acute toxicity (LD_{50}) study of Sunset Yellow azo dye using Wistar 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 Sunset yellow (E110) in dye

Group	Dose (ppm)	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 Sunset yellow dye was estimated to be more than 5000 ppm per body weight of the tested animals. This implies that 50 % or half of the group of tested animals can survive any dose between 1 – 5000 ppm per body weight 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 ppm 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 concentrations of doses administered. Thus, the results in this study agrees with the report of Abad-Fuentes *et al.* (2015) which showed that most of the azo dyes used as food additives and in textiles have very low oral acute (LD_{50}) toxicity as determined using animals such as rats and rabbits (below 10,000 ppm), and dogs (5000 ppm) per their respective body weights.

CONCLUSION

The quantitative assessments of Sunset yellow dye (E110) for oral LD_{50} (acute toxicity) studies and its quantification in some beverages, which are mostly consumed within the metropolitan parts of Katsina (Nigeria) were successfully carried out. Therefore, most of the analyzed beverages are safe for consumption with exception of few ones having concentrations above the maximum permissible limits of 50 ppm as supported by OECD, 2001 (Guideline for the Testing of Chemicals, Acute Oral Toxicity–Acute Toxic). Although, excessive consumption of such beverages containing the dye additives could leads to continuous accumulation of the dye in the body tissues beyond its maximum permissible limits that may results in health issues at long run that include different forms of cancers and also provoking allergic reactions such as asthmatic symptoms.

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