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ACUTE AND SUB-CHRONIC EFFECTS OF SOME SELECTED SYNTHETIC FOOD COLORANTS ON LIVER AND KIDNEY FUNCTIONS IN ALBINO RATS

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ABSTRACT

Synthetic food colorants are widely added to food in order to attract the consumer. Many researchers have incriminated these additives for causing some problems to human health. This research was conducted to determinate the toxic effects of some selected synthetic food dyes (A, B and C) in some biochemical parameters of male albino rats in the acute test. The limit test dose of 5000 mg/kg was administered to four albino rats and then observed individually 1hour post-dosing, and at least once daily for 14 days. Sub-chronic toxicity was evaluated after administering daily oral doses of 1000, 2000, 3000 and 4000 mg/kg body weight, for 28 days to the albino rats. Results of the acute toxicity study indicated that the LD_{50} of the three different standard synthetic color additives (A), (B) and (C) is more than 5000 mg/kg. While in sub-chronic toxicity studies administration of synthetic food colorants, showed significant increases ($P<0.05$), in aspartate amino-transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum urea (SUR) and serum creatinine (SCR) values of rats treated with synthetic dyes when compared to those of the control group. The study showed that it takes more than 5000 mg/kg dose of the dye to kill the tested albino rats and also indicated that the colorants affect the liver and kidney functions.

Keywords: Albino rat, Kidney, Liver, Synthetic food colorant, Toxicity

INTRODUCTION

Food colors are substances which added to food, food products and drink to changes its color to improve the visual quality of food for attraction of the consumer (Saeed. et al., 2018). Food colors are classified as natural and synthetic food colors. Natural Food Color is derived from variety of sources such as seeds, fruits, vegetables, insect and microorganisms without any chemical treatment. In prehistoric times, natural colorants were usually obtained from the sources like flowers, leaves, berries, blossoms, barks and roots.

Synthetic food colors are usually water-soluble chemical substances which have been made in factory and can be used in foods without any further processing. Ponceau 4R, Carmoisine, Erythrosine, Tartrazine, Sunset Yellow FCF are the examples of synthetic food colors (Meenakshi *et al.*, 2017). Synthetic colors are reliable and economical for restoring the original shade of the foods compared to the natural colorants which are expensive and less stable (Nidasaleem *et al.,* 2013) However, the synthetic food colorants showed adverse effect on human health. It has been reported that consumption of synthetic foods color additives could sometimes lead to toxic effects on liver, kidney and testes (Mahmoud, 2016).

The administration of synthetic food colorants decreased the percentage of high density lipoprotein cholesterol (HDL-C), glutathione secretion (GSH), superoxide dismutase (SoD), and plasma immune-system and significantly increased plasma lipid lipoprotein, total cholesterol (LDL-C), lipid peroxidase, blood glucose, plasma urea and creatinine and increased activities of alkaline phosphatase, acid phosphatase, and lactate dehydrogenase (Abdellah *et al.*, 2015). Previous studies investigated the metabolic and toxicological disorders induced by the administration of specific food colorant additives to rats and other mammals. **(**Zraly *et al.*, 2006) The nutritional hazards of synthetic food colors have been detected in the liver and kidney. More attention must be focused on the physiological and pathological effects of colour additives (Ganong, 2016). Previous studies (Al-Shinnawy, 2019) investigated the metabolic and toxicological disorders induced by the administration of specific food colorant additives to rats and other mammals. However, nutritional hazards have been detected in the liver and kidney due to the uncontrolled use of synthetic (El-Malky *et al.*, 2014). Therefore, the present study was planned to demonstrate the effects of three synthetic color additives on the liver and kidney function of albino rats.

MATERIALS AND METHODS

The standard synthetic food colorants used in this study are; (A), (B) and (C) of analytical grade Purchased from a Chemical Store in Katsina state, Nigeria. Distilled – deionized water was used throughout the research.

Experimental Animals

Thirty nine (39) adult male Albino rats weighting (120-150 g) were used in this experiment were purchased from the Animal House of the Department of Biochemistry, Umaru Musa Yar'adua University Katsina, Katsina, State Nigeria.

Acute toxicity test

The limit test dose of 5000 mg/kg was used as stipulated in Organization for Economic Cooperation Development (OECD) guidelines (OECD, 2001). Six male adult rats, each sequentially dosed at intervals of 48 hour, were used for the test. Each rat was kept under observation for the first 2 to 3 hour. Behavioral changes, decrease in body weight was observed for a period of 14 days.

Sub-chronic toxicity studies

Thirty nine (39) rats, divided into four groups, the control group contain 3 rats while the remaining three groups contain 12 rats each. Each group where divided into four containing three rats per cage, were daily administered with different concentrations of the three different standard synthetic color additives (A, B and C) for 28 days. Control group received distilled water, while the other three groups received 1000, 2000, 3000, and 4000 mg/kg body weight. The rats were fasted overnight. On the 29th day, weights were taken and the rats were humanely sacrificed. The blood samples were taken in EDTA containers.

Serum Collection:

Following decapitation of each animal, the blood samples were collected in a clean centrifuge tube placed at 4 ºC for 2 to 3 hours and then centrifuged at 3000 rpm for 15 minutes and the serum was separated and kept at -20 ºC for biochemical analyses.

Serum biochemical determinations

The level of biochemical parameters such as aspartate amino-transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum urea (SUR) and serum creatinine (SCR) were evaluated to determine the function of the liver and kidney of the control groups and the treated rats. All blood biochemical parameters were determined using Auto-chemistry Analyzer in chemical laboratory at General Amadi Rimi Orthopedic and Specialist Hospital Katsina, Katsina State.

Statistical Analysis

The data were analyzed by one-way analysis of variance (ANOVA) using Statistical Package of Social Sciences (SPSS) software. The results were expressed as mean ± standard error (SE). A level of P value less than 0.05 was considered to be significant.

RESULTS

Acute toxicity test

The observation made based on LD_{50} of the three different standard synthetic color additives was estimated to be more than 5000 mg/kg. The dose produced no mortality after 72 hours of observation and up to 14 days period. However, there were significant changes on the behavioral responses of the tested rats after 14 days of observation. Weight loss was recorded in all the 6 treated rats after 14days.

Sub-chronic toxicity Studies

No deaths were observed, but there were significant changes on the behavioral responses at the end of the treatment. Similarly, significant differences in body weight were observed between control and treated groups during this period. The tables (1 – 6) below show the biochemical parameters for the albino rats after sub-chronic toxicity treatment with the three different standard synthetic color additives.

Table 1. Effect of the 'A red' color on the liver function of albino rats

Biochemical Parameters	Control Group	Treatment			
		1000mg/Kg	2000mg/Kg	3000mg/Kg	4000mg/Kg
AST (U/L)	38.15±0.18	44.20±0.22	49.34±0.13	53.45±0.15	59.75±0.13
ALT (U/L)	29.72±0.14	36.38±0.32	45.76±0.14	51.24±0.12	54.84±0.98
ALP (U/L)	23.50±0.45	25.56±0.15	29.21±0.19	32.45±0.44	30.54±0.16

n= mean of three sample ± SEM, = Significant different (P<0.05),
AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase

Table 2. Effect of the 'A' (red color) on the kidney function of albino rats

Biochemical Parameters	Control Group	Treatment			
		1000mg/Kg	2000mg/Kg	3000mg/Kg	4000mg/Kg
SUR (mg/dL)	5.73±0.38	6.34±0.22	6.82±0.12	7.20±0.46	6.98±0.53
SCR (mg/dl)	4.30±0.15	5.02±0.38	5.50±0.63	6.10±0.48	5.89±0.22

n= mean of three sample ± SEM, = Significant different (P<0.05),
SUR= serum urea; SCR = serum Creatinine

Table 3. Effect of the 'B' (yellow color) on the liver function of albino rats

Biochemical Parameters	Control Group	Treatment			
		1000mg/Kg	2000mg/Kg	3000mg/Kg	4000mg/Kg
AST (U/L)	40.05±0.64	55.40±0.26	58.54±0.19	61.57±0.43	60.20±0.75
ALT (U/L)	27.53±0.74	43.82±0.22	45.16±0.47	48.54±0.32	50.94±0.55
ALP (U/L)	25.50±0.45	35.84±0.56	37.29±0.91	40.57±0.23	40.68±0.45

n= mean of three sample ± SEM, = Significant different (P<0.05),
AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase

Table 4. Effect of the 'B' (yellow color) on the kidney function of albino rats

Biochemical Parameters	Control Group	Treatment			
		1000mg/Kg	2000mg/Kg	3000mg/Kg	4000mg/Kg
SUR (mg/dL)	5.70±0.35	5.34±0.67	6.02±0.85	6.30±0.26	6.58±0.44
SCR (mg/dl)	4.00±0.98	4.52±0.38	5.20±0.33	5.54±0.28	5.80±0.62

n= mean of three sample ± SEM, = Significant different (P<0.05),
SUR= serum urea; SCR = serum Creatinine

Table 5. Effect of the 'C' (blue color) on the liver function of albino rats

Biochemical Parameters	Control Group	Treatment			
		1000mg/Kg	2000mg/Kg	3000mg/Kg	4000mg/Kg
AST (U/L)	39.67±0.78	54.70±0.56	56.84±0.22	62.47±0.55	65.50±0.35
ALT (U/L)	28.33±0.54	44.52±0.67	48.06±0.76	52.63±0.29	55.64±0.25
ALP (U/L)	24.20±0.55	36.54±0.76	39.19±0.62	43.27±0.43	42.98±0.35

n= mean of three sample ± SEM, = Significant different (P<0.05),
AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase

Table 6. Effect of the 'C' (blue color) on the kidney function of albino rats

Biochemical Parameters	Control Group	Treatment			
		1000mg/Kg	2000mg/Kg	3000mg/Kg	4000mg/Kg
SUR (mg/dl)	5.50±0.75	6.24±0.57	6.54±0.15	6.84±0.56	7.22±0.56
SCR (mg/dl)	3.95±0.78	4.22±0.45	5.09±0.83	6.22±0.18	5.90±0.44

n= mean of three sample ± SEM, = Significant different (P<0.05),
SUR= serum urea; SCR = serum creatinine

DISCUSSION

The obtained observation in present investigation indicated that the LD_{50} of the three different standard synthetic color additives is more than 5000 mg/kg. This indicates that the test material is likely to be non-toxic or of low toxicity (OECD, 2001). However, higher concentration of the dyes could be toxic in oral formulation. After 28 day of treatment, it is clear that synthetic food colorants treatment resulted in increase in the biochemical parameters of all treated groups comparing to the normal healthy control group presented in tables (1 – 6). The change of these parameters was more evident in those groups treated with synthetic colorants especially 'C' followed by 'B'. However, it was less pronouncing for groups treated with 'A'.

Alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase activities has been increased in all treated groups, but activities of these enzymes were increased remarkably in case of groups treated with synthetic food colorant (C). This study agreed with the findings reported by Mahmoud (2016) who found a significant increase in alkaline phosphatase activity for brilliant blue dye and attributed that to the defect in liver function. Alkaline phosphatase has several physiological functions in bone cells; it splits inorganic phosphates from organic phosphate which is a potent inhibitor of mineralization (Mahmoud, 2016). The significant alterations in the levels of ALT, AST and ALP which are good indicators of liver function suggests that sub-chronic administration of these synthetic dyes altered liver functions of the animals.

Comparing to normal health control, plasma urea was slightly increased in case of the three synthetic colorants treated groups. In groups treated with the three different standard synthetic color additives A, B and C dyes, plasma urea was increased respectively. High increase was observed in uric acid in the groups treated with the synthetic colorants 'C' followed by 'A' and lastly 'B'.

High level of creatinine was also observed in groups treated with synthetic colorants when comparing the control groups. This increase was more pronouncing in case of C colorant treatment. These results are in agreement with the findings reported by Mackenzie *et al.* (2018) and El-Malky *et al.* (2014) who found a significant elevation in urea and creatinine levels in plasma and attributed that to impairment of renal functions. Through this study, the previous data confirmed the destructive effect of the synthetic 'C', 'B' and 'A' colorants on liver and kidney functions. However, this study is also inconsistent with the findings reported by Helal *et al.* (2010) who found that food colorants (both natural: tumeric, carmine chlorophyll and synthetic: fast green, annatto and sunset-yellow) increased serum creatinine levels.

CONCLUSION

The present study demonstrated that it will take more than 5000 mg/kg dose of these selected synthetic colorants to kill the tested albino rats. It is also indicated that these colorants are slightly affecting the liver and kidney functions by considering the increase in the activities of aspartate amino-transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum urea (SUR) and serum creatinine (SCR). It is therefore suggested that excessive use of food colorants should be avoided.

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