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Original Research Article

Impacts of Allura Red (FD&C Red No. 40) on biochemical and physiological properties of albino rats

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ABSTRACT
There are several uses for food additives, including colouring, sweetening, and preservation. However, due to their toxicity, several food additives have been banned from usage. Azo dyes are one of these food additives that are frequently employed in foods as colouring agents. The goal of the current study was to assess the potential effects of an azo dye (FD&C Red No. 40) on a number of physiological and biochemical variables in male albino rats (Rattus norvegicus). The result was that 40 adult male rats weighing 100–110 g were separated into 4 groups, with the first and third acting as controls, the second receiving 50 mg/kg, b.w. of FD&C Red No. 40 for 10 days, and the fourth receiving the same treatment for 40 days. Oral medication was given to all rat groups. The results show that rats treated with FD&C Red No. 40 had significantly lower mean corpuscular haemoglobin concentrations (MCHC), red blood cell counts, and haemoglobin content. In contrast, rats treated with FD&C Red No. 40 showed a measurable rise in their haematocrit (Hct) value, mean corpuscular volume (MCV), activities of the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), glucose level, and serum total protein and globulin. It was evident that giving rats FD&C Red No. 40 led to a wide range of alterations in their physiological and biochemical parameters. In conclusion, further thorough evaluations of azo dye additives in general and FD&C Red No. 40 in particular are necessary.

1. Introduction

To make food and beverages seem better, a variety of food colouring additives are used. Colorant gives food stuff an appealing appearance. A red azo dye by the title of Allura Red – AC is also known as FD&C Red 40. It has the E129 code and is a food colouring. Although it may too be utilised as the potassium and calcium salts, it is typically given as its red sodium salt. These compounds are water-soluble. Its highest absorbance in solution occurs about 504 nm or so. In an azo coupling procedure, 6-hydroxy-2-naphthalene sulfonic acid and diazotized 5-amino-4-methoxy-2-toluenesulfonic acid are combined to create Allura Red, FD&C Red No. 40.

The colour FD&C Red 40 is widely used around the world, mostly for the colour red. Gelatins, puddings, dairy goods, confections, beverages, condiments, and a wide range of other products are all coloured with it. Some problems can be brought on by exposure to various food colours. Foods like drinks and confectionery frequently contain the food colouring FD&C Red 40. However, it is well known to have a number of adverse effects and health risks, just like any other artificial food colour. Kid's hyperactive symptoms, asthma and respiratory issues getting worse, an allergy to aspirin-related intolerance, and the remote possibility of inducing bladder's ulcer in animals are the main adverse effects of the allura dye. Amaranth is another type of red food colouring that is fairly popular, however most people use FD&C Red 40.

Numerous researchers [1-4] investigated the metabolic and toxicological issues brought on by giving rats and other mammals certain food colourant additions. Numerous azo chemicals have been shown to be both carcinogenic in lab animals and genotoxic in short-term studies [5, 6].

The United States allows the use of FD&C Red 40, which is categorised as non-genotoxic [5]. According to United States N.T. Programme, FD&C Red 40 is not positive for salmonella. FD&C Red 40 is not carcinogenic to rats or mice, despite the fact that an impurity in the dye may be decreased to produce an ether-extractable mutagen [7]. One FD&C Red 40 teratology investigation in mice produced no findings [9], while another revealed diminished hyoid ossification [10].

In developing rats, there is evidence of both physical and behavioural harm from FD&C Red 40 [11]. According to a 2001 study by Tsuda et al. [12], mice exposed to very low doses of several azo dyes, such as FD&C Red 40, develop colon DNA severally affect. To reliably estimate the possible threat of these food dyes to mankind, additional research is necessary from various angles.

The goal of the current investigation was to examine how several biochemical and physiological parameters in the albino rats were affected by FD&C Red 40. Additionally, the study aims to ascertain if liver and renal function can be impacted by large dosages of FD&C Red 40.

2. Materials and methods

Materials: The synthetic dye used was di-sodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfonatophenyl)diazenyl] naphthalene-2-sulfonate.

Alternative Names: FD&C Red and Allura Red AC.

The E129 code.

Uses: For colouring, cold-drinks, candies, human and animal medicines, drinks, snacks, baked goods, gelatin desserts, and ice cream are among the items where it is present.

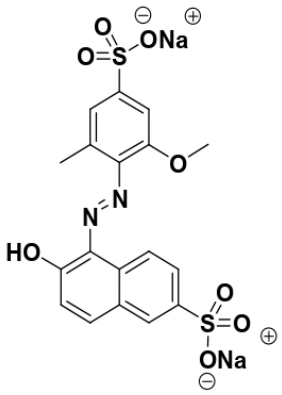

Figure 1. Structure of FD&C Red 40

Animals: In this study, 50 adult (male) albino rats weight ranging between 100 and 110 g were put to the test. The rats were kept in typical lab settings, fed a routine food, and given unlimited access to water. For 12 hours, all rats were hungry. Before treatment, however I let the excess go free into the ocean. They were divided into four groups at random: Twenty rats from the 1st and 3rd groups were used as controls. The 2nd group of 20 rats was provided 45 milli-gram per kilogram body weight per day of FD&C Red 40 orally for two weeks. A sedative of 45 milli-gram per kilogram body weight per day of FD&C Red 40 was also given orally to the fourth group of ten rats for 45 days. All the groups were sacrificed to test the effects of FD&C Red 40 after each treatment interval.

Haematological Studies: The method described in [13] was used to quantify the haematocrit value in a fraction of serum sample that was collected on anticoagulated glass tube. For the purposes of the haematological experimentation, more blood was obtained on EDTA. According to Dacie and Lewis [14], RBC and WBC counts were carried out by making use of an upgraded hemo-cytometer. The Dacie and Lewis' estimation of haemoglobin concentration can be found in Ref. [14]. As per methodology described in Ref. [15], the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC) were determined.

Biochemical Analysis: For each rat, serum sample was taken, allowed to coagulate in neat and clean test tubes, and then subjected to centrifugation at 30500 rpm for 15 minutes. A fraction of the clear superb serum was utilised right away to measure glucose using the Trinder-described enzymatic colorimetric approach [16]. For further investigation, the residual serum was frozen at -20°C. The method outlined by BergMeyer and Bernt [17] was used to estimate the activity of the serum enzymes aspartate aminotransferase (AST) and the alanine aminotransferase (ALT). The Belfield and Golbderg method [18] was used to measure the serum's alkaline phosphatase (ALP) concentration. The urea and creatinine concentration in the serum was calculated using the techniques outlined in References [19, 20]. The estimation of serum's net albumin and protein concentrations followed the procedures outlined in References [21, 22]. The serum globulin was determined using the procedure outlined in Reference [23].

Data Analysis: The student "t"-test was used to statistically analyse the results using the Snedecor and Cochran technique [24].

3. Results and discussion

Table 1's results reveal the changes in various haematological parameters that FD&C Red 40 resulted in the rats that were the subject of the study. RBCs and the mean concentration of corpuscular haemoglobin were considerably decreased ($p \sim 0.01$) in rats feed with FD&C Red 40 for two weeks (MCHC). Additionally, the same dose resulted in the extremely important enhancement ($p \sim 0.02$) in an average of corpuscle while showing a extremely important decrement ($p \sim 0.04$) in the contents of haemoglobin. FD&C Red 40 had no effect on albino rats' leucocyte (WBC) counts or MCHC during the entire experiment.

The information shown in Table 2 showed how treatment with FD&C Red 40 affected enzymatic actions, which are indicators of liver function (LF) in the albino rats. For both of the FD&C Red 40 treatment periods, there was an extremely important increment ($p \sim 0.02$) in ALP, ALT, and AST in the group receiving the drug. The results are shown in Table 3 as shorter- and longer-lived ($p \sim 0.01$) increment in the rat's serum urea, creatinine, and glucose concentration. FD&C Red 40's impact on the net albumin, globulin concentration, serum protein concentration, and the A/g ratio was shown in Table 4.

After receiving the initial dose of FD&C Red 40 for two weeks, total serum proteins and serum globulin concentrations substantially enhanced ($p \sim 0.05$). In other groups and parameters, there were no observable changes of significance.

Table 1. Serum image parameters of albino rats tested with FD&C Red 40.

Groups (*)	R.B.C.s ($\times 10^6$/mm^3)	Hb (g/dl)	Hct (%)	MCV (FL)	MCH (pg)	MCHC (%)	W.B.C.s ($\times 10^3$/mm^3)
After two weeks							
1	7.4±0.03	13.39±0.30	37.40±1.33	45.30±0.20	16.30±0.15	36.50±0.80	3.95±0.10
2	7.3±0.10	13.30±0.40	38.50±1.00	55.50±0.60	16.50±0.30	32.00±0.75	4.75±1.50
After 40 days							
1	7.5±0.10	13.30±0.40	37.50±1.50	45.20±0.80	16.50±0.30	37.25±1.00	4.90±0.25
2	7.2±0.10	12.90±0.20	38.20±1.20	49.20±0.50	16.80±0.10	33.50±1.00	4.80±1.20

(*) The determined parameters were represented in the form: average value ± absolute error of 20 rats. Groups (1 and 3): Control rats. Group (2): Rats feeded FD&C Red 40 for two weeks. Group (4): Rats feeded FD&C Red 40 for 45 days.

Table 2. Influence of temperature with FD&C Red 40 on ALP, ALT, and AST behavior of rats for different groups.

Group No. (**)	AST (U/ml)	ALT (U/ml)	ALP (U/ml)
After two weeks			
1	164.75±0.10	85.55±0.20	177.20±0.50
2	179.80±0.50	135.20±0.90	185.40±0.40
After 40 days			
1	158.25±0.20	87.25±0.10	180.40±0.50
2	187.20±0.50	139.60±0.40	184.50±0.50

(**)(*) The determined parameters were represented in the form: average value ± absolute error of 20 rats. Groups (1 and 3): Control rats. Group (2): Rats feeded FD&C Red 40 for two weeks. Group (4): Rats feeded FD&C Red 40 for 45 days.

Table 3. Influence on serum urea, serum, creatinine, and serum glucose of albino rats of feeded FD&C Red 40 for two groups.

Groups (***)	Serum urea (mg/L)	Serum creatinine (mg/L)	Serum glucose (mg/100 ml)
After two weeks			
1	40.20±1.00	15.00±0.50	85.00±1.20
2	45.25±0.50	21.40±0.50	114.35±0.50
After 40 days			
1	39.20±1.00	15.50±0.90	86.30±0.60
2	50.00±1.00	28.22±0.80	112.25±0.90

(***)(*) The determined parameters were represented in the form: average value ± absolute error of 20 rats. Groups (1 and 3): Control rats. Group (2): Rats feeded FD&C Red 40 for two weeks. Group (4): Rats feeded FD&C Red 40 for 45 days.

Table 4. Influence on serum total proteins, albumin, globulin, and A/g ratio of male albino rats feeded FD&C Red 40 for two groups.

Groups (****)	Serum total protein (g/dL)	Serum albumin (g/dL)	Serum globulin (g/dL)	A/g ratio
After two weeks				
1	8.90±0.10	3.85±0.10	3.35±0.10	1.10±0.25
2	9.00±0.20	3.90±0.20	4.40±0.35	0.78±0.80
After 40 days				
1	7.35±0.20	3.80±0.10	3.50±0.25	1.00±0.50
2	8.45±0.20	3.85±0.15	4.50±0.50	0.88±0.60

(****)(*) The determined parameters were represented in the form: average value ± absolute error of 20 rats. Groups (1 and 3): Control rats. Group (2): Rats feeded FD&C Red 40 for two weeks. Group (4): Rats feeded FD&C Red 40 for 45 days.

The influence of FD&C Red 40 on a few bio-chemical and physio-logical markers in albino rats is the focus of the current investigation. Azo dyes have been examined subcutaneously in rats and tested orally in mice, rats, and canines. Two rat investigations using oral doses of the colour Azo showed a carcinogenic impact [25]. Hematological variables were useful instruments for evaluating injuries brought on by certain drugs. The erythrocyte indices MCV and MCH were best calculated using the RBC counts as the input data. RBC counts are often lower in anaemia of any kind.

Under the administration of FD&C Red 40, significant alterations in the haematological parameters were seen in the current experiment. After 10 and 40 days of treatment, the findings revealed a noticeably lower haemoglobin (Hb), MCHC, and erythrocyte (RBC) count in both groups of treated rats. According to Chakravarty et al. [26], who demonstrated the fall in haemoglobin contents and the net erythrocyte counts at all dose level of dye employed, these changes brought on by FD&C Red 40 may be the result of the food dye's inhibition of erythropoiesis in the bone marrow, which prevents the synthesis of red blood cells.

Ford et al. [27] claimed that the albino rats treated with FD&C Red 40 for the period of approximately 180 days not resulted in any alterations to the haematological studies, in contrast to the data mentioned above. The pronounced disparities between the numerous research trials may be due to dose variances as well as the length of time that food colourants were consumed. In the current investigation, the MCH and total leucocytic count remained constant across all experimental groups. This conclusion was supported by research by Borzelleca and Hallagan [8]. Albino rats treated with tartrazine at levels 2.5, 5.0, and 7.5 mg/kg bw given to rats for the period of three months not impacted the net leucocyte count, as demonstrated by Himri et al. [28].

In the investigation of hepatotoxicity brought on by substances, serum aminotransferase activity are referred to as toxicity markers [29]. Early detection of toxic hepatitis was referred to as an increase in these enzymes' activity. The current study's findings showed that groups treated with FD&C Red 40 experienced a noticeable enhancement in ALP, ALT, and AST activity over the course of the full trial period. An indication of tissue injury has been thought to be an increase in amino-transferase activity in the blood. Similar findings were made by Abdel-Rahim et al. [30], who discovered a considerable rise in serum's ALT and AST levels in rats given red color dye in food for the period of 90 days. Attributing the above modifications in LF to hepato-cellular injury, which consequently led to the discharge of higher than usual

concentration of intracellular enzymes in blood, they claimed that these findings were similar to those made by other studies. The increase in serum's ALP level could be a sign of disruptive hepatic tissue damage brought on by the injection of FD&C Red 40. This finding is consistent with what Chakravarty et al. [26] reported.

According to the report of Amin et al. [9], the albino rats given a high level dose of tartrazine (500 milli-gram per kilogram) for the period of one month showed a considerable enhancement in serum ALT, AST, and alkaline phosphatase activity. These outcomes are consistent with the results of those studies. The current results are consistent with the conclusions arrived by Mekkawy et al. [31], who reported that serum AST, ALT, and alkaline phosphate activities were considerably enhanced in response to two doses of artificial dyes; carmoisine and tartrazine. Additionally, these outcomes are consistent with the outcomes of Aboel-Zahab et al. [23], where he discovered that the albino rats treated with Chocolate hues A and B had higher levels of AST, ALT, and alkaline phosphatase.

The current study revealed a highly substantial enhancement in serum urea concentration in rats given a high level dose of FD&C Red 40 for two weeks followed by a large enhancement. The primary source of ammonia for urea production is protein catabolism [32]. According to Gilman et al. [11], the increased level of urea in this study may have been caused by a rise in nitrogen retention or by impaired renal function. In severe glomerular filtration defects, a discernible rise in serum urea level was seen [32]. An incredibly helpful indicator of kidney function is creatinine, a waste product of the metabolism of creatin [33]. In the current investigation, the FD&C Red 40-treated group's creatinine levels increased in a highly significant way throughout the entire experiment. The current results also line up with information provided in Ref. [34], where the authors noted the substantial enhancement in blood creatinine and urea levels in albino rats given an oral dose of an organic color azo dye for the period of one monts. Additionally, albino rats given either high/low doses of tartrazine showed a considerable enhancement in their serum levels of urea and creatinine, according to Amin et al. [9]. Renal impairment is indicated by an enhancement in serum levels of creatinine and urea [35].

A crucial component of the metabolism of carbohydrates is glucose. Both the digestion of complex carbohydrates and internal body synthesis (gluconeogenesis) result in its formation [33]. The current findings demonstrated that rats given FD&C Red 40 displayed a considerable rise (hyperglycemia) that persisted throughout the experiment. A temporary lack of the pancreas' endocrine functioning that causes hyperglycemia can be attributed to the liver's stimulation of glycogenolysis and gluconeogenesis. Possible explanations for this hyperglycemia include variations in blood sugar levels brought on by foreign substances. These findings support those of Amin et al. [9]. When the albino rats were treated by tartrazine at high (450 milli-gram per kilogram) and at low (15 milli-gram per kilogram) doses for the period of one month, he observed a considerable enhancement in glucose levels.

These findings are consistent with the results reported in Ref. [9] where the authors discovered the considerable enhancement in serum net protein's level when tartrazine was administered to male rats for the period of one month at low and high doses. In this investigation, the serum total protein exhibited a considerable enhancement in albino rats only given FD&C Red 40 for the period of two weeks. Additionally, the results reported in this paper concur with those reported in Ref. [36]. The stimulation of protein biosynthesis to create the precise enzymes needed for all processes is thought to be the reason of the serum protein buildup. The study's rats' serum's globulin fraction showed an increase brought on by FD&C Red 40. The particular rise in globulin level suggested that immunoglobin synthesis, the body's defence against the harmful effects of this artificial food colourant, had increased.

4. Conclusions

The injection of FD&C Red 40 to rats clearly generated numerous changes in the physiological and biochemical markers. In conclusion, further thorough evaluations of azo dye additives in general and FD&C Red 40 in particular are necessary.

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