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PROFILE OF SERUM LIVER ENZYMES OF WISTAR ALBINO RATS FOLLOWING ACUTE ORAL ADMINISTRATION OF A DRUG COLORANT, BRIGHT RED.

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ABSTRACT

The acute in-vivo effect of oral administration of the drug colourant, Bright Red was investigated. Three groups of rats were administered 500mg, 1000mg and 2000mg per Kg body weight of the colourant daily consecutive for three days. The control group was given sham treatment with equivalent volume of distilled water. The effects of the colourant on serum-liver levels of aspartate aminotransferase (EC 2.6.1.1) and alkaline phosphatase (EC 3.1.3.1) were compared with the control group, which received only the diluent for the administration of the test colourant solution. The serum and liver levels of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) increased in a dose related manner. The serum-liver enzymes showed significant elevations ($P< 0.05$) in the three test groups compared to the control group. The results indicate that acute oral administration of Bright Red colourant could be injurious to liver cell integrity.

Key words: Drug colourant, Bright Red, alkaline phosphatase, aspartate aminotransferase, Liver.

INTRODUCTION

The addition of colourants in products have been encouraged by the greater acceptance of naturally coloured products. The use of artificial colourants is rapidly gaining wide industrial applications as most colour products tend to lose their natural shade during processing and storage. The use of colourants in various industrial applications in pharmaceuticals, cosmetics and food products have been documented (Zuckerman, 1964; Kojima, 1978; Bertram, 1989; Garcia et al, 1989; Reyes et al, 1996). The commercially available food and drug colourants are used extensively in preparations such as ice cream; bakery and diary products; toilet extensively in preparations such as ice cream; bakery and diary products; toilet soaps; detergents, lotions, creams, alcoholic and soft drinks in Nigeria. Anliker (1979), reported that under normal conditions of handling and safe use, colourants are generally of low acutetoxicity. However, the reports of Singh et al (1987); Younis et al (1986); Elias et al, (1988); and Agarwal et al (1989) have indicated that some drug and food colourants produce toxic effects.

The conditions of handling, appropriate and safe use of the commercially available colourants in most of the local markets in Nigeria are uncertain, because most of the colourants do not have specifications for safe use. Hence, an acute in-vivo study of the effect of commercially available drug and food colourant, Bright Red was undertaken. This investigation was focused on the liver injury because the liver is the major organ that handles the biotransformation and detoxification of drugs and exogenous substances that get into the body This study examines this possibility by measuring serum liver enzymes in wistar albino rats following acute treatment with the Bright Red colourant.

MATERIALS AND METHODS DRUG COLOURANT

The drug colourant, Bright Red powder (Preema Int. Ltd. London W2, U.K) was purchased in the best conditions of use from a store in Aba, Abia State, Nigeria. The active ingredients in the colourant were sodium chloride, EI24 PONCEAU

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4R; and E102 Tartrazine as indicated by the manufacturer

CHEMICALS AND REAGENTS

The enzymes kits Alkaline phosphatase opt. Kit (Randox Laboratories Ltd, U.K Cat. no AP501) and Glutamic- Oxaloacetic Transaminase kit (Randox Laboratories Ltd, UK. Cat. no AS101) were purchased from the Randox Laboratories limited, United Kingdom in good condition of use. The other chemicals and reagents were of analytical grade except where otherwise indicated.

EXPERIMENTAL ANIMALS

Wistar albino rats of both sexes were obtained from the Animal House, Department of Pharmacology & Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria. They were acclimatized for two weeks and were accustomed to the daily handling and experiment environment. They were housed in plastic cages with stainless steel top. They had free access to feed (Livestock Feed PLC Pfizer Nigeria) and water ad-libitum. The cages were cleaned daily and the sawdust beddings replaced. The temperature of 28 ± 2°C, relative humidity of 60 – 80% and twelve hours natural light/dark cycle were maintained during the period of treatment.

EXPERIMENTATION

Twenty wistar albino rats of both sexes (112-200g) were randomly selected into four groups. Each group contained five rats, which were assigned identification marks. Group 1 constituted the control, Group II received 500mg per kg body weight of the Bright Red colourant respectively. The daily doses were calculated based on the body weight of each rat. The colourant was dissolved in 5mls of distilled water and administered completely. The administration was orally (intubation), daily for three days consecutively, using fresh preparations of the colourant. The control rats were given sham treatment with equivalent volume of distilled water for the same period. The rats had free access to feed and water ad-libitum. At the end of the treatment period, the rats were anesthetized with thiopentone sodium. (Rotex Medica, GMBH, Gernany), given at 60mg/kg body weight.

COLLECTION OF SAMPLES

The blood sample of each rat was collected by heart puncture under thiopentone sodium anaesthesia (Rotex medica, GMBH, Germany) given at 60mg/kg bodyweight. Each blood sample was collected in the test tube and allowed to stand for 30min to clot before being centrifuged. The liver of each rat was carefully excised, blotted dry, weighed and placed in labeled bottles.

PREPARATION OF SAMPLES FOR ASSAY OF ENZYMES

The blood samples were centrifuged using the MSC student centrifuge (Gallenkamp Switzerland, cat. no. CFB 7000 10C) at 5000g for 5minutes. The serum was extracted and stored at 4°C for subsequent analysis of alkaline phosphatase (ALP) and Aspartate aminotranserase (AST). Liver slices of 1.0 -1.6g were homogenizedf using porcelain mortar and pestle in the presence of pure, fine sand grains. The homogenized liver slice were centrifuged with the MSC student Centrifuge at 3000g for 10 minutes and the supernatant collected for enzymes assay.

ENZYME ASSAY

The colorimetric method (Varley, 1980) was used to assay for the enzymes, Alkaline Phosphatase (ALP) and Aspartate aminotransferase (AST). In this method Randox kits (Randox Laboratories Limited, United Kingdom) for each of the enzymes was used in the assay. The company standard specified instructions for each kit was used.

The Hitachi UV/Visible spectrophotometer (Hitachi Japan, HACH DR/3000, model 19600-00) was used to measure the absorbance at 546mm and 405nm wavelengths for aspartate aminotransferase and alkaline phosphatase enzymes respectively.

STATISTICAL ANALYSIS

Data were computed and analysed using

group comparison. Statistical significance was determined using the student-t-test and was considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Table 1 shows the body weight of both the control and the treated rats. The results indicate that Bright Red Colourant caused a dose related loss in body weight of the rats. The control rats, however, indicated a gain in body weight. Bright Red colourant at 2000mg body weight caused a significant loss ($P<0.05$) in body weight when compared to the initial body weight of the corresponding rats. While the loss in body weight at 500mg or 1000mg per kg body of the colourant was not significant ($P>0.05$) when comparedf to the initial body of the weight of the corresponding rats. Possibly, the loss in the body weight could be attributed to slight reduction in feed intake, and the moderate diarrhoea observed during the treatment period. While the slight reduction in feed intake may have been caused by satiety due to the volume (5mls) of colourant administered and the increased water intake observed during the treatment period. The liver weight of the control and the treated rats are presented in Table 2. the results indicate an increase in the liver weight of the rats expressed as percentage of their final body weight of the corresponding rats in a dose related manner, which were not significantly different ($p>0.5$) from the control. Increased liver weight in the treated rats

TABLE 1 – Effect of Acute Oral Administration of Bright Red Colourant on Body weight

Treatment Doses (mg)	Initial Body Weight (g)	Final Body Weight (g)	Change
Control	145.4 ± 0.9	146. 4 ± 2.1	1 ± 1.3 (0.69 ± 0.6)
500mg per kg body wt.	129.6 ± 6.0	129.4 ± 5.3	0.2 ± 0.1 (0.15 ± 0.1)
1000mg per kg body wt.	183.5 ± 18.5	181.5 ± 16.5	2 ± 2. 0 (1.10 ± 0.7)
2000mg per kg body wt.	173.5 ± 5.5	167.8 ± 7.4	*5.7* 2.9 (3.31 ± 1.0)

Data show pooled values (n=5), Mean ± S.E.M.
Change in body weight is expressed as absolute values.
Values in parentheses are percentage change in body weight
Wt = Weight, *, $P<0.05$

TABLE 2 – Effect of Acute Oral Administration of Bright Red Colourant on Liver Weight of Rats.

Treatment (mg)	Initial Body Weight (g)	Final Body Weight (g)	Liver Weight (g)	Liver Weight as percentage of final body weight (%).
Control	145.4 ± 0.9	146.4 ± 2.1	4.51 ± 0.19	3.08 ± 0.36
500mg per kg body weight	126.6 ± 6.0	126.4 ± 5.3	4.06 ± 0.21	3.14 ± 0.14
1000mg per kg body weight	103.5 ± 18.5	181.5 ± 16.5	5.95 ± 0.01	3.28 ± 0.17
2000mg per kg body weight	173.5 ± 5.5	167.8 ± 7.4	5.62 ± 0.31	3.35 ± 0.11

Data show pooled values (n = 5), Mean ± S. E. M.

Table 3 – Effect Acute Oral Administration of Bright Red Colourant On the Serum-Liver Aspartate Aminotransferase

Treatment Doses (mg)	Serum Level (U/l)	Liver Level (U/l)
Control (distilled water)	107 ± 12.1	163 ± 6.4
500mg per kg body weight	107 ± 2.3	*174 ± 6.8
100mg per kg body weight	109 ± 2.9	*179 ± 2.3
200mg per kg body weight	*110 ± 1.3	*189 ± 1.0

Data show pooled values (n = 5), Mean ± S,E.M.
* indicates significantly different P<0.05 with respect to the Control.

Table 4 – Effect Acute Oral Administration of Bright Red Colourant On the Serum-Liver Alkaline Phosphatase

Treatment Doses (mg)	Serum Level (U/l)	Liver Level (U/l)
Control (distilled water)	106 ± 14.3	117 ± 13.7
500mg per kg body weight	*147 ± 16.8	*157 ± 12.5
100mg per kg body weight	*153 ± 12.5	*169 ± 17.0
200mg per kg body weight	*172 ± 11.1	*179 ± 18.5

Data show pooled values (n = 5), Mean ± S,E.M.
* indicates significantly different P<0.05 compared to the Control.

could be a possible consequence of hepatic cellular oedema and hyperplasia.

Robbins et al (1984) reported that oedema is associated with transudation and accumulation of fluid in the soft tissues of the body. Retention of sodium in the hepatic extracellular tissue compartment could result in oedema. Sodium chloride is a constituent of the Bright Red colourant and its hepatic retention may be responsible for water retention in the liver causing oedema and subsequent increase in liver weight.

Casarett (1975) reported the presence of protein in the cytoplasm of liver cells with a high binding affinity for azo dyes. The binding of Bright Red colourant by the liver cells could cause lipid and fatty accumulations in the liver which could possibly cause liver hyperplasia in the treated rats. Ramachandani et al (1992) also reported lipid peroxidation of ultrastructural components of rat liver induced by Metanil Yellow and Orange II, which the Bright Red colourant could possibly have caused in this study.

Table 3 shows the effect of Bright Red colourant on the serum and liver levels of aspartate aminotransferase (AST). The results indicate a dose dependent increase in both the serum and liver levels of aspartate aminotraferase. Bright Red colourant at 2000mg per kg body weight caused a significant increase (P<0.05) in the serum level of AST compared to the control. While the liver level of AST at all the treatment doses were significantly higher (P<0.05) compared to the control. The effect and liver of Bright Red colourant on the serum levels of alkaline phosphatase (ALP) is presented on Table 4. The results show a dose-related increase in both the serum and liver level of ALP. Bright Red colourant at 500mg, 1000mg and 2000mg per kg body weight caused significantly higher (P<0.05) level of alkaline phosphatase in the serum and liver compared to the control. Damage of the liver cells is associated with leaking out of enzymes from the liver into the blood (Mayne, 1990). This could have been the case in this study.

Alkaline phosphatase is a sensitive indicator of

early intrahepatic and extrahepatic bile obstruction (Varley, 1980). Similarly aspartate aminotransferase is a sensitive indicator of liver cell damage for both acute and chronic hepatocelluar injury (Varley, 1980).

Wilma et al (1976) indicated that tests for liver enzymes are a useful index in monitoring the chemical situation of the liver. While Handler et al (1994) further established that initial assessment of hepatobiliary damage can be accompanied by measuring serum activities of liver associated enzymes viz: alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase. Hence, the significantly high levels of serum-liver aspartate aminatransferase and alkaline phosphatase probably are indicative of the injurious effect of Bright Red colourant on the liver at the treatment doses. The effect could be more pronounced with repeated or prolonged use of the colourant.

This could impair normal liver functions such as synthesis, metabolism and detoxification. Impaired liver synthetic process will certainly affect important body developmental and physiological processes and could cause health complications. The misuse of Bright Red colourant could constitute health risks to the general public. Surveillance in its use in local preparations should go hand in hand with good pharmaceutical manufacturing practice.

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