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ACUTE IN-VIVO GASTROINTESTINAL EFFECT OF FOOD COLOURANTS, BRIGHT RED AND EGG YELLOW IN RATS

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ABSTRACT

The acute in-vivo gastrointestinal tract effect of food colourants, Bright Red and Egg Yellow given to rats by oral intubation was investigated.

Two sets of experiments involving fourty albino rats of both sexes, divided into eight groups of rats were carried out. The rats were either administered Bright Red or Egg Yellow colourant at 500mg, 1000mg or 2000mg per kg body weight daily, consecutively for three days. The control groups were given sham treatment with equivalent volume of distilled water. Oral administration of either Bright Red or Egg Yellow colourant at 1000mg or 2000mg per kg, caused mild suppression of appetite (anorexia), stimulation of polydipsia, diarrhoea, reduced agile movements and colouration of skin and urine which was not observed in the control rats. Gross examination revealed marked ulcerative lesions and haemorrhage on the antra of stomach of the rats given the colourants at 2000mg per kg body weight.

The observed histomorphological changes in the stomach of rats treated with the Bright Red colourant at higher doses (1000mg/kg and 2000mg /kg) were mucosal degeneration, aggregate of inflammatory cells, transmural submucosal oedema, and focal mucosal lesions. While the histomorphological changes in the stomach of rats treated with the Egg Yellow colourant at 1000mg /kg and 2000mg /kg were oedema, cellular degeneration, superficial mucosal erosion and submucosal inflammation. These histomorphological changes were absent in the control. The results indicate that Bright Red and Egg Yellow oral ingestion could cause gastric ulcerations, diarrhoea and other gastrointestinal disturbances and should be used cautiously in food preparations.

KEYWORDS: Food colourants, Bright Red, Egg Yellow, Gastrointestinal Tract.

INTRODUCTION

Colourants are added to products to improve their appealing appearance and appetizing qualities. The documented use of coulourants have been provided by Zuckerman and Kohnstam (1964); Kojima (1989); Garcia et al (1989); and Reyes et al (1996). In some West African countries (Nigeria and Cameroon), their use in domestic and other local preparations is on the increase. The commercially available colourants are used in the preparation of alimentary products viz: alcoholic drinks, wines, soft drinks, ice cream, bakery and diary products, snack foods and some herbal preparations. The reports of Zivkovic et al (1985); Yournis at al (1986); Singh at al (1987); Elias et al (1994); FAO/WHO expert committee reports on food additives (1991); Agarwal et al (1994); Babu and Shenolikar (1996); Koutsogeorgopoulou et al (1998) and Ching et al (2002) indicate that some food and drug colourants produce toxic effects. Most of the commercially available food colourants in the local markets in Nigeria neither have documented literature on their toxicological evaluation nor specifications for safe use or daily acceptable intake values. The over ingestion of these colourants could likely contribute to some of the disturbances of the gastrointestinal function of their consumers. The aim of this study was to investigate in rats, the possible acute in-vivo gastrointestinal tract toxic potentials of the food colourants, Bright Red and Egg Yellow.

MATERIALS AND METHODS

Food colourants

The colourants, Bright Red powder and Egg Yellow powder (Preema Int. Ltd. London W2, U.K.) were purchased in the best conditions for use from a store in Aba, Abia State, Nigeria. The active ingredients in the colourants as indicated by the manufacturers showed that the Bright Red powder contained sodium chloride, E124 PONCEAU 4R, and E102 Tartrazine while the Egg Yellow powder consisted of E110 Sunset Yellow FCF, E102 Tartrazine and Sodium chloride.

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 with the approval from the animal protection recommendation and ethetical committee of the University. They were acclimatized for two weeks and were accustomed to daily handling and experimental 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. Their cages were cleaned daily and the sawdust beddings replaced. The temperature of 28 $\pm$ 2^{0}C, relative humidity of 60 – 80% and twelve hours natural light/dark cycle were maintained during the period of treatment.

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EXPERIMENTATION

Two sets of experiments were performed. In the first set of experiments twenty wistar albino rats of both sexes (150-200g) were randomly allocated into four groups. Each group contained five rats, which were assigned identification marks. Group one constituted the control. Groups Two, Three and Four received 500mg, 1000mg and 2000mg per kg body weight of the Bright Red colourant respectively through the oral route. In the second set of experiments, twenty wistar albino rats of both sexes (150-200g) were randomly selected into four groups of five rats in each group. Group One constituted the control, while groups Two, Three and Four received 500mg, 1000mg and 2000mg per kg body weight of Egg Yellow colourant through the oral route respectively.

The colourants were dissolved in 5mls of distilled water and administered completely. The oral administration was by intubation given daily for three days consecutively using fresh preparations of the colourants. The control rats were given sham treatment with equivalent volume of distilled water for the same period. The rats were carefully monitored for overt manifestations of toxic reactions, which included aggressiveness, reduction in agility, salivation, convulsion, sleeping, mouth breathing, breathing difficulties, and restlessness. All the rats survived till the end of the treatment period.

The rats were sacrificed by stunning, quickly exsanguinated and the abdominal cavity dissected. The stomach, ileum and large intestine were excised and examined for gross morphological changes using a hand lens. The stomach and ileum were fixed in 10% formal saline.

HISTOPATHOLOGICALSTUDIES

Sections of tissues of the stomach and ileum fixed in 10% formal saline were cut and processed for histopathological studies as described by Drury and Wallington, 1967. The prepared slides were stained for histopathological changes using the Haematoxylin-Eosin (H and E) routine method of Baker and silverton, 1985.

RESULTS AND DISCUSSION

The rat treated with Bright Red and Egg Yellow colourants at the dose levels of 500mg, 1000mg and 2000mg per kg body weight exhibited symptoms of acute toxic reactions. These included mild suppression of appetite (anorexia); stimulation of polydipsia; diarrhoea; reduced agile movements; colouration of skin and urine. These symptoms were not observed in the control rats. Although the cause of the anorexia was not investigated, anorexia observed in the colourants treated animals is more probably due to their destructive effects on the "feeding center" or stimulation of the "satiety center" in the hypothalamus. The polydipsia evident in the treated rats may be attributed to the osmotic (salt) effect. Sodium chloride is a constituent of the colourants. Besides, Egg Yellow colourant contains E110 sunset yellow FCF, which is a trisodium salt (Zuckerman and Kohnstam 1964). Similarly, the diarrhoea observed during the treatment period was probably associated with osmotic (salt) effect causing increased gastrointestinal tract motility.

Gross examination of the stomach of rats treated with either Bright Red or Egg Yellow colourant at 2000mg per kg body weight indicated marked ulcerative lesions at the antra regions, haemorrhage and oedema. No gross morphological changes were seen in the ileum and large intestines of the rats treated with either Bright Red or Egg Yellow colourants at any of the dose levels. However, the surfaces of the ileum and the large intestine were stained with the respective colourants with which the rats were treated. The stomach, ileum and large intestines of the control rats (given sham treatment with equivalent volume of distilled water) showed normal gross morphology

Ulcerative gastritis is multifactorial. The damage to the stomach mucosa of the rats caused by Bright Red or Egg Yellow colourants at the higher doses in this study, probably occurred by direct contact of the colourants with the gastric mucosa.

Histopathological examination of the stomach of the rats treated with Bright Red or Egg Yellow colourants revealed a variety of degenerative inflammatory and proliferative lesions. However, the stomach and ileum of the rats which were given sham treatment with distilled water alone (control) showed normal morphology (Fig. 1).

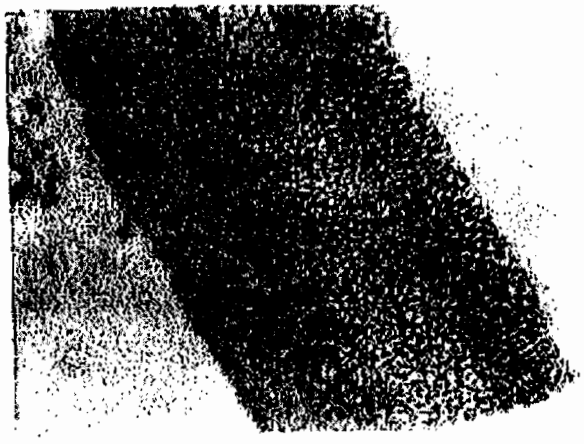

Fig. 1: Control section of rat stomach administered distilled water alone, showing normal histological morphology. (H & E x 40)

At 500mg per kg body weight, Bright Red colourant caused mucosa degeneration in the stomach, with no observable histological changes in the ileum. At 1000mg per kg body weight Bright Red colourant caused mucosal degeneration, oedema, and aggregation of submucosal inflammatory cells in the stomach, while only mild mucosal inflammation was observed in the ileum. Transmural submucosal oedema, inflammatory cells and focal mucosal lesions (Fig. 2) were observed in the stomach of the rats treated with Bright Red colourant at 2000mg per kg body weight. While moderate acute inflammatory changes (enteritis) occurred in focal areas of the ileum. The histomorphological changes observed in the stomach of the rats treated with Bright Red colourant at 2000mg/kg

confirmed that the ulcerative lesions, haemorrhage and oedema observed with gross examination of the excised stomach were due to the treatment with the colourant.

Fig. 2: Section of rat stomach after acute oral treatment with Bright Red at 2000mg per kg showing transmural oedema and aggregation of inflammatory cells (H & E x100)

At 1000mg and 2000mg per kg body weight, Egg Yellow colourant caused oedema, cellular degeneration and superficial mucosal erosions as well as submucosal inflammation in the stomach (Fig. 3). However, there was no histopathological changes in the ileum. No histopathological changes were observed in stomach and ileum of rats with Egg Yellow colourant at 500mg per kg body weight. The observed histological features in the treated rats were dose – related and markedly different from those in the tissues of the control rats.

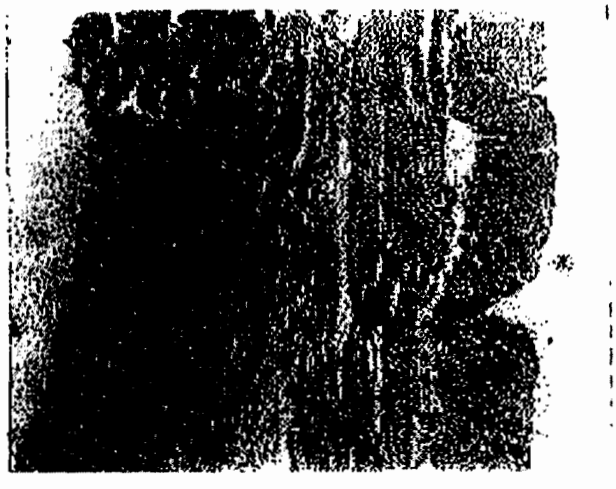

Fig. 3: Section of rat stomach after acute oral treatment with Egg Yellow colourant at 1000mg per kg showing oedema of the submucosa, cellular degeneration and superficial mucosa erosions (H & E x 100)

The results show that the commercial food colourants, Bright Red and Egg Yellow are not completely harmless as is claimed by the manufacturers. They are capable of causing gastrointestinal toxic reactions. The symptoms of toxic reactions observed with acute oral administration of higher doses (1000mg or 2000mg per kg) of either Bright Red or Egg Yellow suggest that the colourants can pose some health problems following repeated ingestion of the colourants.

Although the dose of 1000mg and the maximum dose of 2000mg per kg body weight used in this study may appear to be high, there is no record of quantification of the actual amount of these colourants in local food preparations. Privileged information to the authors indicate that the present difficult economy in Nigeria, and the high cost of tin tomato is forcing many public food and local eating houses to substitute unripe paw-paw fruit blended with Bright Red colourant for tomatoes in stew. The Bright Red colourant is therefore used ultimately to provide the red tomato colour. It could be difficult to determine how much of the Bright Red colourant would be required to simulate the tomato redness in the stew, vis-à-vis the amount consumed. Though experimental results using laboratory animals as model are usually interpreted with guarded caution in terms of extrapolation to man, the gastric lesions, diarrhoea and other gastrointestinal disturbances observed in the study can occur in man with repeated ingestion of these colourants over a short period. It is therefore concluded that the food colourants, Bright Red and Egg Yellow investigated in the study are likely to cause potential health problems to the general public who routinely and naively consume these colourants in the various food preparations.

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