EVALUATION OF SUNSET YELLOW INDUCED LIVER DAMAGE BY SELECTED SERUM TRANSAMINASES LEVELS

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ABSTRACT
We have evaluated toxic effect of graded doses of synthetic food colour sunset yellow (SY) in male albino rats by measuring activity of ALT, AST and ALP in their serum following 90 days exposure to four groups of rats with (0.0%) control, 0.25%, 0.50%, and 3% SY. The results obtained showed significant dose-dependent increase (p<0.01) in enzyme activities with respect to the control group. Dose dependent elevation of SY on the transaminase was substantiated by varying percentage. After 90 days exposure with mid and high concentration of SY, total bilirubin activity also increased significantly; 12.50% (p<0.01) and of 25% (p<0.01) respectively. Histopathological studies showed liver necrosis. Present study revealed the synthetic food additive SY causes hepatocellular damage.

Keywords –Sunset yellow FCF, liver transaminases, alanine amino transferase, aspartate amino transferase, alkaline phosphatase, bilirubin, histopathology

INTRODUCTION
Food colouring can be used in food, drugs, or cosmetics. Everyone is sensitive to the colour of food. Appetite stimulation or dampened has almost direct relation to our reaction to colour. Romans recognized that “people eat with their eyes” as well as with their palates. Nowadays market is inundated with artificial colouring agents. Those meant for dyeing textiles are being added to food products (Mathur 2005). Their toxicity has hardly been tested. The unregulated use of additives has led to many death and is posing a serious public health concern. Two types of colour added to the food are natural and synthetic. Natural colours are permitted in any food and in any amount like saffron and turmeric. According to Amin and Al-Shehri (2018) the food additives agents can be divided into preservative, flavoring, stabilizer, emulsifiers, nutritional additives and sweetners agents.

Red, yellow, blue and green are 4 basic synthetic colours (Mekkay 2001). The prevention of Food Adulteration Act (PFA) permits eight synthetic colours to be added to food. These include ponceau 4 R, carmoisine, erythrosine, tartrazine, sunset yellow, indigo carmoisine, brilliant blue and fast green (Anon. 2003). Sunset yellow is widely used in the food products, drugs, cosmetics and pharmaceuticals in India. It is an incredibly strong synthetic colourant (dye) containing azo functional groups and aromatic ring structure harmful to human health. Clinically chronic exposure to sunset yellow caused hyperactivity, allergies, urticarial, rhinitis, nausea, vomiting, indigestion and abdominal pain (Jamiloki 2014).

Sunset yellow (SY) FCF is a water soluble synthetic orange-red coloured azo-dye that has been used in pharmaceuticals, cosmetics and food industry field. Chemically SY (E110 or FD and C Yellow No. 6: CI No. 15985) is principally the disodium salt of 1- (4 sulphonephenylazo) - naphthol-6-sulphonic acid with solid in the name of orange red, that was first used in 1929. In past few years, use of some food colour including SY was banned in United States and Japan owing to its mutagenicity but used freely in India. The accepted daily intake (ADI) for man expressed on a body weight basis is the amount of a food colour that can be taken daily in the diet, even over a lifetime, without risk. An ADI is allocated only to substances for which adequate short term and long term toxicological results are available. As per norms of international research and the recommendations of the Codex Committee on food additives and Contaminants (CCFC), intake of dye is under control of ADI (acceptable daily intake).

The average daily consumption (ADI) of this dye has undergone many changes over the years. In 1982 and 1994, ADI was accepted as 0-2.5mg/kg body weight by FAO/WHO Expert Committee on Food Additives (JECF) and
the Scientific Committee on Food (SCF). In the year 2009 European Food Safety Authority Panel (EFSA) decided to reduce the ADI, by an extra uncertainty factor of 2.5 to 1 mg/kg bw/day and to make ADI temporary for 2 years. Joint FAO/WHO Expert Committeee on Food Additives (JECFA) and EFSA ANS panel (2014) has established the maximum permitted level of SY for different types of food. The permissible limit of SY is 300 mg/kg food whereas, for water-based flavored drinks, the safety limit allowed is 100 mg/kg drink. After this rate or limit was reduced to 0-4mg/kg (Colakoglu and Muhammer 2021). The aim of this work was to study hazardous effects of commercial SY on histopathology and biochemical changes in the liver of male Swiss albino rats.

**MATERIALS AND METHODS**

**Experimental animals:** Healthy male albino rats weighing 150-184g used for the present study were fed on standard laboratory feed and fresh water *ad libitum*.

**Sunset yellow FCF:** Sunset (E110 or FD and C Yellow No.6: CI No. 15985) is a synthetic azo-dye, water soluble, and orange-red coloured powder and chemically is principally the disodium salt of 1-(4 sulphonylphenyl azo) - naphthol-6-sulphonic acid with solid in the name of orange red, that was first used in 1929. SY purchased from the local market was manufactured by Bush Company, Chennai India. It was mixed with pellet diet and then fed to the albino rats belonging to various experimental groups. Experimental doses were decided according to human ADI permitted in India (PFA 1980), WHO/FAO Expert Committee Guidelines and the published work from ITRC, Lucknow.

**Experimental design:** The animal divided into 4 groups were having 10 animals each. The control (Group I) received only the normal pellet diet, whereas the animals of group 2, 3 and 4 were administered SY mixed with pellet diet at the dose level of 0.25%, 0.50% and 3.0% respectively for a period 90 days.

**Collection of tissues and blood samples:** The animal were sacrificed after 90 days. The liver was quickly excised and fixed in Bouin's fluid for histological observation. 5 micron thick sections were stained by haematoxylin and eosin (H and E). Biochemical investigation were carried out on liver tissue and serum collected from the experimental animals.

**Liver function:** Serum AST and ALT were determined by method Bergmeyr and Bernet (1974). Serum ALP was estimated by King and King (1954). Serum bilirubin was determined by the method descreibed by Walter and Grade (1970).

**Statistical Analysis:** Data on biochemical values were statistically calculated using Student ‘T’ test (Lipsen and Feigl 1970) and analysis of variance. Results were expressed as mean ± S.E.

**RESULT**

**Body weight:** Albino rats of 0.25% 0.50% SY treated groups showed a non significant reduction in their body weight of 1.63% and 2.17% (P = ns) while it decreased (18.47%) significantly in 3% group when compared with control (Table 1).

**Liver weight:** Liver weights increased significantly in groups exposed to higher doses of dye (Table 1). In general, the lower dose of SY was slightly toxic while its higher doses were moderately toxic in albino rats.

**Histopathological Investigation:** In comparison to control male rat liver dose dependent histopathological alternations in the liver of dye exposed mice were; perportal necrosis with yellow brown coloration, lymphocytes infiltration around the blood vessels and slight congestion in blood vessels and increase in the number of Kupffer cells along with dilatation in different areas of hepatic tissue. The centrolobular and mid-zonal necrosis was accompanied with hypertrophy and hyperplasia of hepatocytes (Fig. 2 and 3). Many folds increase in number of swollen were observed in hepatocytes alongwith with nuclear degenerative changes. Binucleate conditions were also observed in few hepatocytes (Fig 4).

<table>
<thead>
<tr>
<th>Treatment of SY after 90 days</th>
<th>Body weight in gm (Mean ± S.E)</th>
<th>% decrease in body weight</th>
<th>Liver weight in gm (Mean ± S.E)</th>
<th>% increase in liver weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>184± 8.98</td>
<td></td>
<td>5.89± 0.94</td>
<td></td>
</tr>
<tr>
<td>0.25%</td>
<td>181±0.49</td>
<td>-1.63%</td>
<td>5.94 ± 0.08</td>
<td>+0.84%</td>
</tr>
<tr>
<td>0.50%</td>
<td>180± 0.70</td>
<td>-2.17%</td>
<td>7.90 ±0.24**</td>
<td>+34.12%</td>
</tr>
<tr>
<td>3.0%</td>
<td>150± 7.07**</td>
<td>-18.47%</td>
<td>6.65 ± 0.30**</td>
<td>+12.90%</td>
</tr>
</tbody>
</table>

(**P<0.01 from control). Each value is the mean of 10 different values and their errors.
Histopathological Investigation: In comparison to control male rat liver dose dependent histopathological alterations in the liver of dye exposed mice were; periportal necrosis with yellow brown coloration, lymphocytes infiltration around the blood vessels and slight congestion in blood vessels and increase in the number of Kupffer cells along with dilatation in different areas of hepatic tissue. The centro-lobular and mid-zonal necrosis was accompanied with hypertrophy and hyperplasia of hepatocytes (Fig. 2 and 3). Many folds increase in number of swollen were observed in hepatocytes along with nuclear degenerative changes. Binucleate conditions were also observed in few hepatocytes (Fig 4).

Fig. 1: Liver section of control rat showing central vein (CV), radially arranged hepatic cords (HC) and sinusoids (SS) between hepatic cells x 100 H and E.
Fig. 2: Microphotograph of 0.25% SY treated rat liver showing the binucleated (BN) hepatocytes x 200 HE.
Fig. 3: Microphotograph of 0.50% SY treated rat liver showing the swollen hepatocytes (SH) x 200 HE.
Fig. 4: Microphotograph of 3.0% SY treated rat liver showing the binucleated (BN) and swollen hepatocytes (SH) x 200 HE.
Biochemical investigation: The data indicates a profound change in the biochemical profile of adult male rat treated with different doses of SY for 90 days depended manner when compared with control (Table 2). There was significant dose dependent increase in ALT, AST, ALP and total bilirubin values of dye treated mice.

Table 2. Effect of sunset yellow on Serum biochemical parameters in Male Albino Rats after 90 Days.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Serum Biochemical Parameters</th>
<th>Control (Mean± S.E.)</th>
<th>0.25% SY (Mean± S.E.)</th>
<th>0.50% SY (Mean± S.E.)</th>
<th>3.0% SY (Mean± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ALT</td>
<td>32.7±8.53</td>
<td>51.9±1.98</td>
<td>72.1±9.13**</td>
<td>91.5±14.21**</td>
</tr>
<tr>
<td>2</td>
<td>AST</td>
<td>143±21.95</td>
<td>179±3.6</td>
<td>208±29.67**</td>
<td>326±1.59**</td>
</tr>
<tr>
<td>3</td>
<td>ALP</td>
<td>134±20.40</td>
<td>155±0.85*</td>
<td>228±15.92*</td>
<td>224±6.15**</td>
</tr>
<tr>
<td>4</td>
<td>Bilirubin</td>
<td>0.8±0.05</td>
<td>0.83±0.02*</td>
<td>0.9±0.03**</td>
<td>1.0±0.02**</td>
</tr>
</tbody>
</table>

(*P<0.05 and **P<0.01 from control). Each value is the mean of 10 different value and their error

DISCUSSION

The reduction in food intake decreased body weight of treated mice when compared with control (Table 1). It was either related to systemic toxic effect or of a local effect of SY on gastrointestinal tract. Brantom et al. (1987) however, reported mean body weight of the green’s dye treated mice similar to control. The liver weights of SY treated mice increased possibly due fatty accumulation in the hepatocytes (Gaunt et al. 1972). In the present investigation exposure to SY for 90 days caused necrosis, karyolysis and cytolysis to the hepatic cells as also reported by others in the experimental animals exposed to hepatotoxicants (Sharma et al 1983, Mathur et al. 2003).

The determination of ALT and AST activities is the most important useful tool in the hepatotoxicity studies. These enzymes form a link between carbohydrates and protein metabolism. Liver has a largest concentration of both enzymes. The damage to the tissue may release these enzymes into the blood stream (Chaudhary 2020). A significant increase in the activities of serum ALT and AST were recorded in male albino rats of low, medium and high dose groups (Table 1), as also reported by other workers (Sharma et al. 1992, Bansal and Bhatnagar 2005). Increase activities of ALT and AST are considered as a sensitive indicator of overall liver health and liver cell injury (Chaudhary 2006). The elevation of serum aminotransferase activities might be due to liver, kidney or heart tissue damage and increased permeability of cell membrane or synthesis or decreased catabolism of aminotransferase.

Tameda (2005) mentioned that release of high levels of specific enzymes into blood stream is dependent on both the degree and the type of damage exerted by toxic compound administration. Webner (2003) reported increase in ALT and AST levels on account of their release from damaged liver. Same results were found in our experiment. Similar results were reported by others in azo dyes exposed rats (Aboel-Zahab et al. 1997, Amin et al. 2010, Saxena and Sharma 2014).

ALP present in many tissues is not an organ specific enzyme. It is closely connected with lipid membranes in the canaliculair zone of liver, and so any interference with the bile flow, whether intra or extrahaepatic, lead to an increase in ALP (Chaudhary 2006). Bilirubin which is an endogenous compounds has been used to evaluate chemically induced hepatic injury. Bilirubin is the major bile pigment derived from the metabolism of haemoglobin and other porphyrin compounds. In our results elevated levels of bilirubin has been observed in SY treated groups. Keeping in view of our findings and of others; We suggests revised ADI of SY for man as 0-2mg/kg body weight.

CONCLUSION

In conclusion, although being approved for use in the food and pharmaceutical industries, synthetic food colour SY can pose health risk. From result of the current study, it is evident that SY can affect adversely and alter functions of vital organs (e.g., liver and kidney). SY not only increase the risk of hepatocellular damage at higher dose, but also induces cancer. It is also confirmed from this study that the liver is the major target organ of SY toxicity. In our study, the safe level of this artificial food colour was low dose (0-2 mg/kg body weight). Therefore, it is necessary to create public awareness regarding the ill effects of synthetic food colour and encourage consumption within ADI range. These result suggest that public should minimize the use of fast food...
containing colour additive, because SY are very harmful. The
effect of daily intake may not express in one or two years,
but it take 5, 10 or 15 years. Then it may become too late.

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