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A REVIEW ON EFFECTS OF FOOD ADDITIVES: ALLURA RED (AZO DYE) AND SODIUM BENZOATE (PRESERVATIVE)

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ABSTRACT

Food additives are chemical substances which are added intentionally to food to upgrade its quality. Allura red (food color) and sodium benzoate (food preservative) are most commonly used food additives in food industries, pharmacy as well as in cosmetics for different purposes like, preserving and coloring. Experimental data of last ten years on allura red and sodium benzoate caused liver, kidney, reproductive, brain toxicity in various animal models have been reviewed. Allura red and sodium benzoate cause Histological changes in liver, kidney, stomach, DNA damage, behavioral changes, biochemical changes, haematological alterations and oxidative stress at various dose levels.

Keywords: food additive, allura red, sodium benzoate, toxicity

INTRODUCTION

Humans consume lots of structurally different chemical substance such as preservative, color, flavorings, sweeteners, antioxidants, stabilizers and emulsifiers **[1]**. Food additives are mixed to food in small amount intentionally during manufacturing and processing to improve taste, color, quality, nutrient value, to maintain stability and freshness and to prevent decomposition of food by microorganisms **[2-5]**. Food additives intentionally mixed to food for specific purpose (directly) and mixed during storage, packing and processing (indirectly) **[6]**. There are many short term and long term adverse effect of food additives. They can affect at molecular and cellular level. There can be skin reaction like eczema, dermatitis, urticaria, and gastrointestinal problem like nausea, vomiting, diarrhea, central nervous system

problem like headache, somnolence, hyperactivity and behavioral disorders. There can be allergic reaction, anaphylaxis, reproductive toxicity, developmental toxicity, degenerative disease and may be carcinogenic **[1, 2, 5, 6]**.

AZO DYE ALLURA RED AND ITS EFFECT:

Synthetic and naturally derived food colorants are used in variety of food products. These can be any pigment, dyes or natural resources (mineral, vegetable and animal) or chemically manufactured. Due to more attraction and stability mainly synthetic colorants are used **[7, 8]**. 60 to 70 % synthetic dyes used in industry are azo dyes. These dyes are mainly used in area of pharmacy, plastics, in laser optical systems, photodynamic therapy, dying of textiles, leather, paper, food and cosmetics. Azo dye have main functional di nitrogen group with combining azo alkyl or aryl radicals **[9]**.Toxic effect is not from native dye agent but it is mainly from toxic derivative formed during degradation process such as benzidine, toluene, aniline and naphthalene. Many azo dyes have mutagenic, carcinogenic and genotoxic properties. Many cancers such as bladder, spleen, hepatocellular carcinoma are linked with azo dyes **[10, 11]**.

Allura red is a food colorant synthetic azo dye. The molecular formula of Allura red is $C_{18}H_{14}N_2Na_2O_8S_2$ (2-hydroxy-1-(2-methoxy, 5-methyl, 4-sulphonatophenylazo)-naphthalene-6-sulphonate), molecular weight is 496.3g/mol. It is disodium salt and soluble in water. Allura red is mainly used in dairy products, cotton candy, snacks, puddings, sauces, cereal, bakery products, orange soda, gelatins, drugs and cosmetic products **[8, 12]**. Allura red is European Union additive 129 (E129) or FD & C Red No. 40 or Food Red 17 and its Acceptable Daily Intake (ADI) is 0-7 mg/kg body weight according to SCF (European Union's Scientific Committee for Food) and JECFA **[7]**. Various scientists have reported that azo dye such as Allura red, Sunset yellow cause changes in liver whichinclude fibrosis, elevation of Kupffer cell number, cytoplasmic vacuolization of hepatic cells, necrosis and degeneration in hepatocytes. Vacuolation of endothelial lining glomerular tuft, vacuolation of epithelial lining renal tubules, necrosis, inflammation, damage in glomeruli with thickening of Bowman's capsule occur in kidney **[11, 12]**. At pH 7.0 Allura red stimulate amyloid-like aggregate formation of hen egg white lysozyme (HEWL). Allura red at concentration of 0.03-15.0 mM HEWL constructs amyloid fibrils due to increase in β-sheet structure of HEWL and reduce its α-helical structure. Amyloid fibrillation of HEWL occurs due to interactions between negatively charged

sulfate group of Allura red and positively charged amino acids (arginine, lysine and histidine) of HEWL **[13]**. Despite absence of Allura red in many genotoxicity assays its *in vivo* presence is found in stomach gland, colon and lung of mice. This dye accelerates reticuloendothelial tumor appearance, so found carcinogenic **[14]**. Red 40 and sunset yellow dyes produce amino naphtholsulphonate compound which are neither carcinogenic nor genotoxic **[15]**. In a 3 to 24 hours sampling time there are no specific DNA damage in colon in comet assay done *in vivo* rats with a dose ranging 10 to 1000mg/kg. Increase in migration of length of stained DNA was the indicator of DNA damage **[16]**. Increase in frequency of MnPCE and DNA damage in colon, liver and stomach are statistically insignificant in orally treated verses control animals with Allura red. This indicates that Red 40 does not induce aneugenicity or clastogenicity *in vivo* in mice **[17]**. No statistically significant difference was observed in Allura red treated verses control animal group in numbers of hedgehogs or DNA damage and no clinical sign or deaths was observed in both groups. Frequency of MNPCEs (micronucleated polychromatic erythrocytes) in positive control group was increased in comparison with the negative control group (carboxymethylcellulose sodium). Increase in MF (mutant frequencies) of cII genes in liver and glandular stomach tissue are insignificant in Allura red treated group. MFs increase in liver and slightly but insignificantly induce in stomach tissue in positive control group (DMBA) **[18]**.

In a observational study to see DNA damaging effect with any concentration (1250 to 5000ug) of amaranth and Allura red at a temperature 28^0c verses 37^0c. There was no significant damage at 28^0c but significant damage was observed at 37^0c. Both azo dyes have optimal action on yeast cells at 37^0C so believed that both dyes start DNA damage at concentration above 1250lglml at 37^0C **[19]**. N-methyl-D-aspartate receptor subunit (NR2A, NR2B) and nicotinic acetyl-choline receptor subunit (nAChα4, nAChα7 and nAChβ2) protein expression in hippocampi.In male group, expression of NR2B and nAChβ2 increased but expression of nAChα4 decreased. In female group main effect of artificial food color and additives are decreased expression of NR2B. Expression of NR2A and nAChα7 are insignificant in both male and female. Some excitotoxicity in neuron was observed due to significantly increase in NR2B expression (positive effect on memory and learning) in male rats and negative effect on memory and learning in female due to decrease expression of NR2B. This study result further increase attention towards effect of artificial food color and additive between

two genders **[20]**. A renal histopathology caused by Allura red on male rat which are changes in glomerulus, proximal and distal convoluted tubules, Bowman's capsule. Allura red also cause renal corpuscles atrophy, tubular necrosis, distortion of glomeruli, capillary tuft compression lead to decrease GFR (glomerular filtration rate) **[21]**.

Author	Animal model	Area	Dose and duration of azo dye	Observation
[12].	**Male albino rat**	**Blood**	**400mg/kg Allura red and brilliant blue for 60 days**	**Elevation in triglycerides, LDL-c, cholesterol, uric acid, urea, creatinine, serum total protein, globulin, total bilirubin, albumin, AST, ALP, ALT and WBC values.** **Reduce body weight of rats and decrease in RBC, Hb and HDL-c**
[21]	**Male albino rat**	**Blood**	**50mg/kg, bwt. Allura red for 10 and 40 days**	**Increase in mean corpuscular volume, haematocrit value, serum total protein, globulin, glucose level, serum aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase activities as well as decrease in R.B.Cs count, haemoglobin and MCHC**
[11].	**Male rat**	**Kidney and liver**	**7mg/kg/dayAllura red and 2.5mg/kg bwt sunset yellow for 4 weeks**	**Increase in urea, creatinine, uric acid, AST and ALT in kidney and liver. Decrease in total antioxidant level and increase in MDA level. Bcl_2 expression level decrease but COX2 expression level elevates in kidney tissue.**
[22].	**Male albino rat**	**Brain and kidney**	**200mg/kg bwt.Allura red for 8 weeks.**	**Reduction in Neurotransmitter contents such as serotonin, 5-hydroindoleacetic acid, GABA, histamine.** **Increase urea and creatinine in serum.** **Reduction in MDA and GSH content in kidney and brain tissue.**
[23].	**Male rat**	**Medial prefrontal cortex**	**7mg/kg bwt. and 70mg/kg bwt.Allura red for 42 days.**	**Low dose group show depletion in number of glial cells. High dose group show depletion in volume of median prefrontal cortex, number of neurons, glial cells, length of dendrites and number of spines per dendritic length.**
[24].	**Tenebrio molitor larvae**	**Body weight**	**1, 0.1, 0.01, 0.001, 0.0001g/kg of dry fodder for 21days tartrazine, Allura red and indigo carmine**	**All dyes cause decrease in body weigh larvae.** **No death observed.**

SODIUM BENZOATE AND ITS EFFECTS:

For preservation of food both natural and chemical substances are used. Sodium benzoate is a synthetic food additive which is most frequently used as preservative against yeast, fungi and bacteria. Sodium benzoate is used in pharmaceutical,

cosmetics, jam, soft drinks, cakes, muffins, biscuits, juices, jellies, bakery products, tomato sauce, salad dressings, wine, beer and olives **[25, 26]**. Sodium benzoate is the sodium salt of benzoic acid with molecular weight of 144.1g.mol and a white crystalline, odorless compound with chemical formula $C_7H_50_2Na$. E number of sodium benzoate is E211. Acceptable daily intake of sodium benzoate is 5mg/kg and it is soluble in ethanol and water **[27]**. Toxic effect of sodium benzoate (SB) includes cell death, cancer, cytotoxic and mutagenic effect **[25, 26]**. Effect of sodium benzoate on T and B lymphocytes at dose 1000µg/ml repress the multiplication of the Concanvalin A and lipopolysaccharide stimulated splenocytes. Sodium benzoate repealed the activation of T-cell against allogeneic MHC antigen. SB causes reduction in splenocytes number because cell cycle at G_1 phases **[28]**.

When zebrafish larva was exposed at 100ppm of sodium benzoate concentration pericardial and yolk sac edema and tail bending was observed. After 96 hours of exposure 100% mortality and reduction in hatching rate was seen **[29]**. Mixture of sodium benzoate and sunset yellow causes ring chromosome, end-to-end union, chromatid break, centric fusion break and splitting of chromosome. When concentration of mixture increase then level of DNA damage elevates in liver tissue **[30]**. When pregnant rats were treated with sodium benzoate (0, 0.5, 1, 1.5mg/ml) increase in body weight, food and water consumption was observed. Perinatal death increase but death decrease in litter. DNA breaks in mother and fetal liver tissue observed in SB treated groups **[31]**. Sodium benzoate (0, 0.5, 1.0, 1.5 and 2.0mg/ml concentration) induced micronucleated cell, chromosome break, gap and sister chromatid separation in chromosome, chromosome abnormality (micronuclei, lagging, anaphase Bridge, stickiness) **[32]**.

Food additives such as sodium benzoate, mono sodium glutamate and tartrazine elevate the level of glucose, insulin, serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, creatinine, urea, albumin, total cholesterol, total lipid, triglycerides, HDL-cholesterol, LDL-cholesterol, lipid peroxidase, glutathione peroxidase and electrolytes (sodium, potassium and chloride) and decrease the level of total protein. Chlorophyllin and sunset yellow cause insignificant effect on liver function, kidney function, lipid profile and oxidative stress markers **[33, 34]**. Male rats treated to sodium benzoate (200mg/kg), sodium nitrite (80mg/kg) and their combination for 8 weeks caused significant elevation in the level of ALT, AST, ALP, albumin, bilirubin, urea, creatinine and LPO in plasma but decrease the level of CAT,

GSH, SOD and total protein in plasma. Significant increase in the expression of P53 (tumor suppressor gene) in kidney and in liver was also observed. Histology of kidney showed peri-glomerular edema, glomeruli shrink, devolution of renal tubule lining, pyknotic nucleus of renal tubules, necrosis, cellular vacuolation, lumen of tubules contains eosinophilic hyaline casts. Histology of liver showed increase in number of hepatic cells, abnormal and congested central vein and haemorrhage in portal region, disappearance of normal blood sinusoid and central vein, dilated blood sinusoid, cellular infiltration and necrosis **[35, 36]**.

[37].	**Swiss male albino mice**	**Brain**	**0.56, 1.125 and 2.25 mg/ml for 4 weeks.**	**No effects observed on body weight and water intake. Significant effects were observed on memory and motor coordination. Level of MDA increase and level of GSH decrease significantly in brain. Non significant alteration of acetylcholine esterase activity was observed.**
[38].	**Male albino rat**	**Blood**	**0.2mg, 0.5mg, 1mg and 10 mg of sodium benzoate, 0.1mg, 0.2mg, 0.5mg and 10mg of ascorbic acid, Carbonated soft drink for 21 days.**	**Insignificant difference observed in White blood cell count, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Significant difference observed in RBC countand haemoglobin concentration (10mg SB and 0.2 mg AA+ 10mg of SB) but other groups showed insignificant difference. Haematocrit value increase (10mg of AA) as compared to other groups. Platelets value elevates.**
[39].	**Male wistar albino rat**	**Blood**	**20mg/kg of aspartame, 20mg/kg of sodium benzoate, 20mg/kg of sodium nitrite and 20mg/kg of sodium sulfite for 30 days.**	**Level of ALT, ALP and AST in serum elevate in treated groups. CAT and LPO level in serum increase but GPx level decrease in treated groups.**
[40].	**Male rat**	**Blood**	**1% solution of glyphosate, saccharin, sodium benzoate and 1% solution of glyphosphate with saccharin.**	**Reduce body weight in treated groups was observed. Reduction in the weight of thymus, duodenum and large intestine. Increase the weight of lungs. Elevate the level of total protein, albumin, globulin, ALP, AST, ALT, total bilirubin, urea, glucose and cholesterol in serum. RBC and WBC count decrease.**
[41].	**Albino rat**	**Blood, liver and kidney**	**Sodium benzoate (10mg, 75mg, 100mg, and 750mg) and sunset yellow (5mg, 20mg, 50mg, and 200mg) for 12 weeks.**	**Level of haemoglobin, haematocrit, RBC, WBC and platelet decrease and level of MCV, MCH and MCHC increase in treated groups.Congestion in renal blood vessel, vacuolation in renal tubules and glomerular tuft, protein cast in lumen of tubules in kidney tissue was observed. Congestion in central vein, apoptosis and necrosis in hepatic cell, portal edema, fibroblasts proliferation in portal triad in liver tissue was also noted.**
[42].	**Rabbit**	**Liver and kidney**	**100 and 200 mg/kg sodium benzoate.**	**ALT and AST level in serum increases. Histology changes in liver include increase in Kupffer cell, shrinkage of nucleus, dense chromatin, necrosis and Damage renal tubules, congestion, intrusion of inflammatory cells, damage in nuclei and basement membrane.**
[43].	**Mice**	**Liver and kidney**	**150, 300 and 600 mg/kg sodium benzoate for 4 weeks.**	**LPO and GSH level high and Catalase level low in kidney tissue but LPO, CAT, SOD, GSH level no change in liver tissue. Histology of liver show inflammation in hepatocytes and mild interstitial inflammation. Kidney show normal**

				glomeruli and tubules and mild to moderate inflammation.
[44].	**Male albino rats**	**Liver**	**1gm./kg bwt. of sodium benzoate for 365 days.**	**Histology changes are necrotic foci of liver cell, enlarged vacuolated cytoplasm, increase in number of lipid droplets, collagenous fibers increase, decrease polysaccharides and total protein.**
[45].	**Male albino rat**	**Male reproductive system**	**250mg/kg of carmoisine, 15mg/kg of mono sodium glutamate and 50mg/kg of sodium benzoate.**	**Level of testosterone hormone, follicle stimulating hormone, luteinizing hormone, gonadotropin releasing hormone in serum decreased. Level of LPO increased and level of GPx, CAT and SOD decreased in serum.**

CONCLUSION

In various animal models alteration in the biochemical parameters of various organs and histological changes in the tissue associated with usage of food additive suggest adverse effects and negative health issues. The present review highlights the effects caused by consumption of sodium benzoate and allura red and it also reflects the toxicity caused in various organs on the different species depending on the dose and exposure to the food additive.

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