Toxicology International, *31(4) : 567-577; 2024. DOI: 10.18311/ti/2024/v31i4/44140*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.co.in/index.php/toxi



Changes Caused in Liver Parameters in Swiss Albino Mice by Food Colourant (Allura Red) and Preservative (Sodium Benzoate)

Preeti Sharma, Lata Shahani* and Mahima Swarnkar

Department of Zoology, IIS (Deemed to be University), Gurukul Marg, SFS, Mansarovar, Jaipur - 302020, Rajasthan, India; lata.shahani@iisuniv.ac.in

Abstract

Food additives are chemicals that are purposefully added to food to improve its quality. The most widely used food additives in the food industry, pharmacy and cosmetics field are Allura red (a food colour) and sodium benzoate (a food preservative), both of which are employed for various functions such as colouring and preserving. In this study, liver parameters were examined in Swiss male mice after 30, 45 and 60 days of treatment with synthetic Allura red colour and sodium benzoate preservative. Seventy-two male Swiss albino mice (25-30 gm) were divided into four groups with 6 mice per group for each autopsy interval. The first group was control-treated (water), the second group was Allura red treated (172.2 mg/kg body weight), the third group was sodium benzoate treated group (123 mg/kg body weight) and the fourth group was Allura red + sodiumbenzoate treated (86.1 mg/kg + 61.5 mg/kg body weight) for 30, 45 and 60 days. Mice were sacrificed after the 31st, 46th and 61st day and blood samples were taken for serological tests to study SGPT, SGOT, ALP, total protein and serum albumin. The liver was also removed for histological studies of control, sodium benzoate, Allura red and combination groups. The data obtained reveals a noticeable significant increase in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein and albumin in treated mice as compared to control groups. The microscopic study of the liver showed the effect of all dose treatments on the structure of liver cells. It caused fatty degeneration of hepatocytes, congestion of the central vein and necrosis. In conclusion, it was clear that the administration of Allura red, sodium benzoate, and combination dose (AR+SB) in mice caused changes in liver parameters.

Keywords: Albino Mice, Allura Red, Blood, Food Colour, Liver Parameters, Sodium Benzoate

1. Introduction

Humans intake a mixture of different compounds with various structural characteristics, including stabilisers, emulsifiers, antioxidants, artificial flavours, colours and preservatives[1]. Food additives are purposely added to foods in minute quantities during manufacturing and processing to enhance flavours, colour, quality and nutritional content, maintain stability and freshness and stop microbes from decomposing food[2-5]. Food additives are purposely combined with food for a specific purpose (directly) and combined during storage, packing and processing (indirectly)[6]. Long- and short-term adverse effects of food additives are many. They can influence

**Author for correspondence*

Article Received on: 22.05.2024 Revised on: 10.08.2024 Accepted on: 13.09.2024

cells and molecules. There may be gastrointestinal issues like nausea, vomiting and diarrhoea, skin reactions like eczema, dermatitis and urticaria as well as problems with the central nervous system including headaches, somnolence, hyperactivity and behavioural disorders. There could be a degenerative condition, an allergic reaction, anaphylaxis, reproductive toxicity, developmental toxicity and perhaps carcinogenic effects[1,2,5,6].

Food colour additives are any pigment, colourant, dyestuff and other substances that give colour to food, beverage, medicine and makeup products[7]. About 16.4% of products in India include colours that are not approved[8]. These dyes are mostly utilised in the fields of pharmaceuticals, photodynamic therapy, plastics, textile and leather dyeing, laser optical systems, food, cosmetics and paper[9]. Both artificial and organicallysourced food colours are utilised in food products but the most widely used food colours are synthetic organic compounds. Customers are fascinated by products by enhance their natural colour with synthetic food colouring 60 to 70 per cent of synthetic dyes used in industry are azo dyes. Azo dye combines an aryl or alkyl radical with a primary functional di-nitrogen group[9]. The hazardous effect is not from the native dye agent, but it is mainly from toxins formed during the degradation process such as benzidine, aniline, naphthalene and toluene. Many azo dyes have mutagenic, carcinogenic and genotoxic properties[12,13]. Humans may be at risk because Allura red binds to Human Serum Albumin (HAS). Fluorescence, UV absorption and circular dichroism can all be used to analyse how Allura red and HAS interact with one another[14]. Colour causes liver cell injury due to the production of highly free radical molecules by metabolic activation of the compound[8]. According to scientists, azo dyes like Allura red and sunset yellow can cause cytoplasmic vacuolisation of hepatic cells, an increase in the number of kupffer cells, necrosis, and damage to the liver cells. When male rats treated with Allura red kidney showed changes in the bowman's capsule, glomerulus, proximal and distal convoluted tubules, tubular necrosis, and capillary tuft compression leading to a decrease in glomerular filtration in the rat [13, 15]. Allura red causes an elevation in creatinine, uric acid, urea, total protein, albumin, globulin, total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, glucose level, haematocrit value, mean corpuscular volume and decrease in body weight, haemoglobin, RBC, a decline in the values of serotonin, GABA, and histamine which are contents of neurotransmitter [15,16].

Both natural and artificial materials are used to preserve food. A synthetic food ingredient called sodium benzoate is most typically used as a preservative against bacteria, yeast and fungi. Pharmaceuticals, cosmetics, jam, soft drinks, tomato sauce, juices, biscuits, muffins, jellies, salad dressing, beer, wine and olives are among the products that contain sodium benzoate. The hazardous effects of sodium benzoate include cytotoxicity, mutagenicity, cancer and cell death[17,18]. Sodium benzoate elevates the level of MCV, MCH, MCHC, insulin, SGOT, SGPT, ALP, glucose, total bilirubin, albumin, creatinine, urea, cholesterol, lipid, HDL and LDL cholesterol, triglycerides, LPO, GPx, and decrease the level of haemoglobin, haematocrit, RBC, WBC, platelet, total protein, SOD, CAT, GSH. Increased level of expression of P53 (tumour suppressor gene) is seen in the liver and kidney. An increase in hepatic cell count, aberrant and clogged central vein, haemorrhage in the portal region, loss of the normal blood sinusoid and central vein, dilated blood sinusoid, necrosis, and cellular infiltration were all seen in the liver's histology. The kidney's histology revealed peri-glomerular oedema, shrunken glomeruli, degeneration of the renal tubule lining, necrosis and cellular vacuolation[20-24].

2. Materials and Methods

2.1 Experimental Materials

Allura red AC (E129) is a red food colour and a mono-azo colour used as a synthetic food colour. It is used in powder mixes, candies, jellies, icings, frozen treats, spices, colouring beverages, sauces, gelatin products and dairy products. Allura Red AC consists mainly of disodium 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfonato-phenylazo)-2-naphthalene- sulfonate and subsidiary colouring matter together with sodium chloride and sodium sulphate as the principal uncoloured components[11,15] WHO.

Sodium benzoate is a white crystalline, odourless compound and is the sodium salt of benzoic acid with a molecular weight of 144.1g.mol and chemical formula $C_7H_50_2Na$. The E number of sodium benzoate is E211. 5mg/kg is its acceptable daily intake, and it is soluble in ethanol and water[19]. Allura red and sodium benzoate used for the treatment was obtained from Sigma-Aldrich.

2.2 Experimental Animals

Seventy-two male Swiss albino mice, weighing 25-30 gm. were used for the experiment. The approval for performing

experiments was taken by the ethical committee of IIS (deemed to be University), Jaipur (IAEC- Reg No- 1689/PO/S13//CPCSEA). The mice were acclimatised for seven days before dose administration.

2.3 Experimental Design

The animals were divided into four groups of 72 mice each. (6 mice for each autopsy interval).

Group1. Control group animals were given water (vehicle) for 30, 45 and 60 days respectively.

Group2. Allura red (172.2 mg/kg) treated group for 30, 45 and 60 days respectively. Group3. Sodium benzoate (123 mg/kg) treated group for 30, 45 and 60 days respectively.

Group4. Allura red (86.1 mg/kg) and sodium benzoate (61.5 mg/kg) treated groups for 30, 45 and 60 days respectively[25].

The effects of these groups on biochemical parameters were examined after three different experimental periods of 30, 45 and 60 days.

2.4 Blood Sample Collection

After 30, 45 and 60 days of treatment, the animals were sacrificed, and the blood sample was collected through the heart on the 31st, 45^{th,} and 61st days of exposure. Serum was collected for the study of liver parameters.

2.5 Histopathological Analysis

Each sample of liver tissue was removed after 30, 45 and 60 days of treatment, washed with ice-cold saline solution and then fixed in 10% formalin for histological analysis. All samples were cleaned with tap water, dehydrated in alcohol, clarified in xylene and embedded in paraffin. Hematoxylin and eosin were used to stain a 5µm thin section. A light microscope was used to examine the liver slide to identify any deformities in liver tissue.

2.6 Statistical Analysis

Results were compiled using the softer ware SPSS by one-way ANOVA test. Data were expressed as mean ± standard error.

3. Result and Discussion

The results of liver parameters are shown in the figures.

3.1 Figure 1

Alanine Aminotransferase (ALT) or Serum Glutamic Pyruvic Transaminase (SGPT) on different groups of mice after treatment for 30, 45 and 60days. p>0.05 as compared to control (non-significant), p<0.05 as compared to control (significant*), and p<0.01 as compared to control (highly significant).

Mice treated with AR and SB for 30 days showed a significant increase (P<0.05) in ALT values. No-significant (P<0.05) increase was observed when mice were treated with a combination dose (AR+SB) for 30 days. When a combination dose was given orally for 45 and 60 days, ALT values showed a highly significant

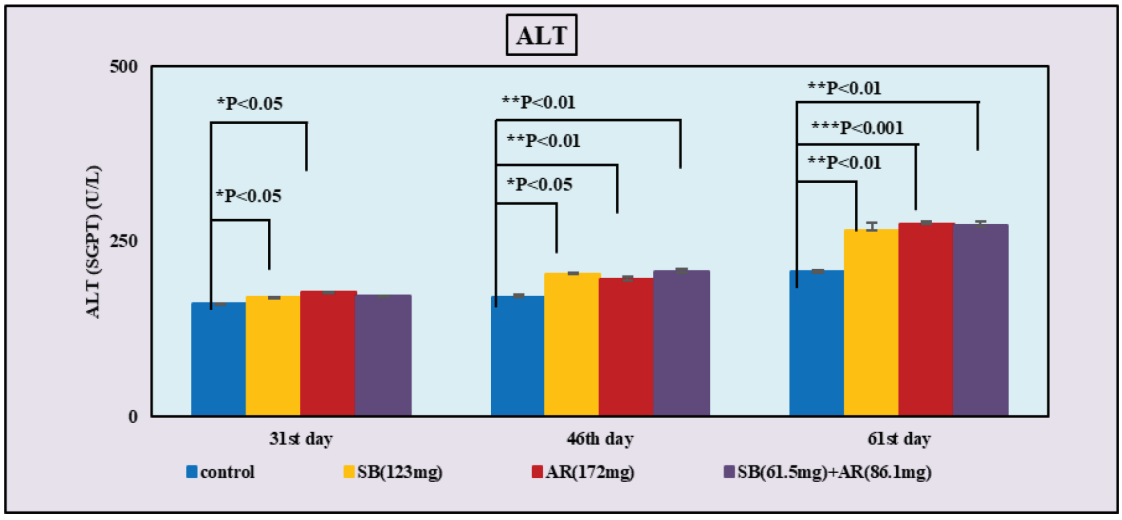


Figure 1. Alanine aminotransferase (ALT) or serum glutamic pyruvic transaminase (SGPT).

increase (P<0.01) as compared to the control group. Mice treated with AR and SB showed a significant increase for 45 days respectively. A highly significant increase was observed in the values of ALT after 60 days of AR and SB treatment.

3.2 Figure 2

Serum Aspartate Aminotransferase (AST) or Serum Glutamate Oxaloacetate Transaminase (SGOT) activity on different groups of mice after treatment for 30, 45 and 60 days. p>0.05 as compared to control (non-significant), p<0.05 as compared to control (significant*), and p<0.01 as compared to control (highly significant).

Mice treated with AR, SB, and a combination dose (AR+SB) for 30 days showed a significant increase (P<0.05) in AST values. when AR and combination dose (AR+SB) were administered orally, AST values showed a highly significant (P<0.01) and SB showed a significant increase (P<0.05) after 45 days. Administration of all doses resulted in a highly significant increase (P<0.01) observed after 60 days.

3.3 Figure 3

Serum Alkaline Phosphatase (ALP) activity on different groups of mice after treatment for 30, 45 and 60 days. p>0.05 as compared to control (non-significant), p<0.05

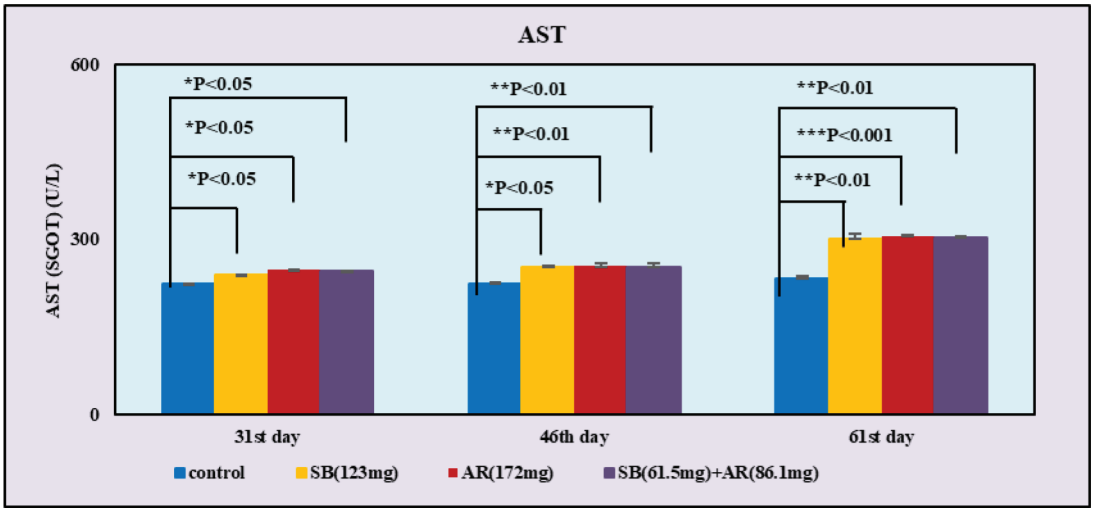


Figure 2. Serum aspartate aminotransferase (AST) or Serum glutamate oxaloacetate transaminase (SGOT).

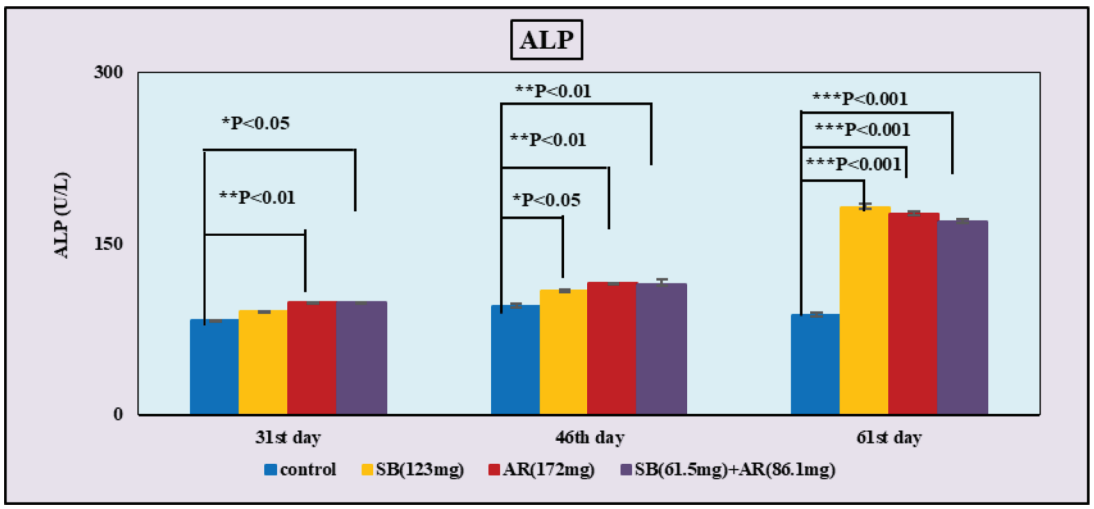


Figure 3. Serum alkaline phosphatase (ALP) activity on different groups of mice.

as compared to control (significant*) and p<0.01 as compared to control (highly significant).

A non-significant increase was observed in ALP values when mice were treated with SB for 30 days. When SB was administered orally for 45 days, ALP showed a significant increase (P<0.05) as compared to the control group. A highly significant increase (P<0.01) was observed in ALP values when mice were treated with AR for 30 and 45 days. When a combination dose (SB+AR) was given for 30 and 45 days, a significant increase (P<0.05) and a highly significant increase (P<0.01) were observed respectively. When all doses were given for 60 days, ALP values showed a highly significant increase (P<0.001) as compared to control groups.

3.4 Figure 4

Serum total protein activity on different groups of mice after treatment for 30, 45, and 60 days. p>0.05 as compared to control (non-significant), p<0.05 as compared to control (significant*), and p<0.01 as compared to control (highly significant).

A non-significant increase was observed in protein levels after 30 days of SB dose treatment. When AR and combination dose were administered orally for 30 days highly significant increase (P<0.01) and a significant increase (P<0.05) were observed in protein level. When SB, AR and combination dose (AR+SB) were given for 45 and 60 days, protein level showed a highly significant increase (P<0.01) as compared to the control group.

3.5 Figure 5

Serum albumin activity on different groups of mice after treatment for 30, 45 and 60 days. p>0.05 as compared to control (non-significant), p<0.05 as compared to control (significant*), and p<0.01 as compared to control (highly significant). A significant increase was observed in albumin levels after 30 days at all dose treatments. When SB and combination dose (AR+SB) were given for 45 days, albumin level showed a significant increase (P<0.05) as compared to the control group. A highly significant increase was observed in albumin levels when mice were treated with all dose treatment after 60 days.

3.6 Histopathological Analysis of the Liver

Effect of Allura red and sodium benzoate alone and in combination on the liver in mice after 30 daysof treatment. The control group showed normal hepatic architecture with the normal Central Vein (CV), Portal Area (PA), Hepatocytes (H), and Sinusoidal Space (SP); Allura red, sodium benzoate and a combination showing fatty degeneration of H and more sinusoidal space.

Effect of Allura red and sodium benzoate alone and in combination on the liver in mice after 45 days of treatment. The control group showed normal hepatic architecture with the normal CV, PA, H and SP, allura red, sodium benzoate and a combination showing fatty degeneration of H, Vacuolar Degeneration (V) with congestion of Central Vein (CV), necrosis near CV and more sinusoidal space.

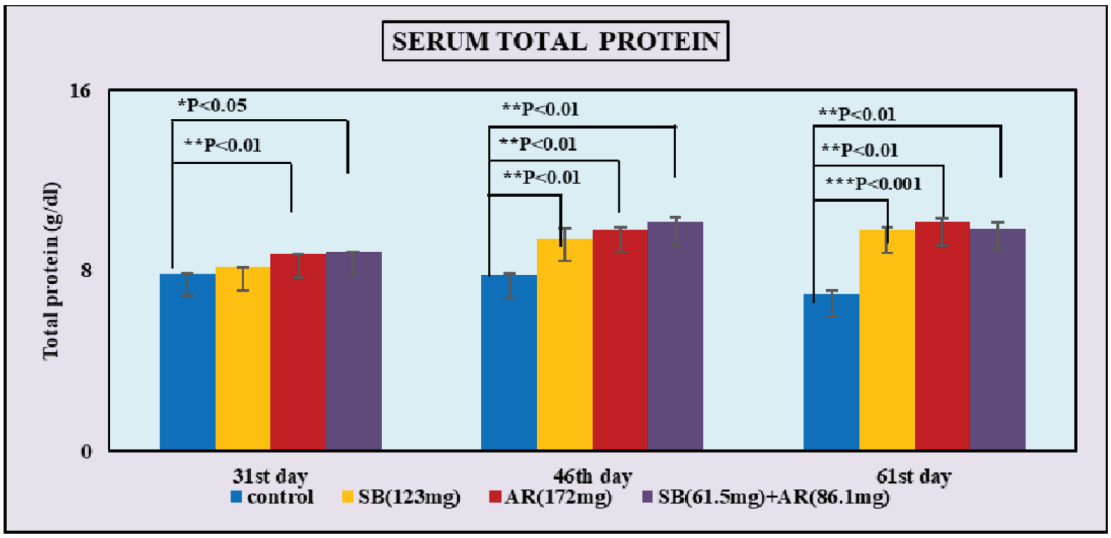


Figure 4. Serum total protein activity on different groups of mice.

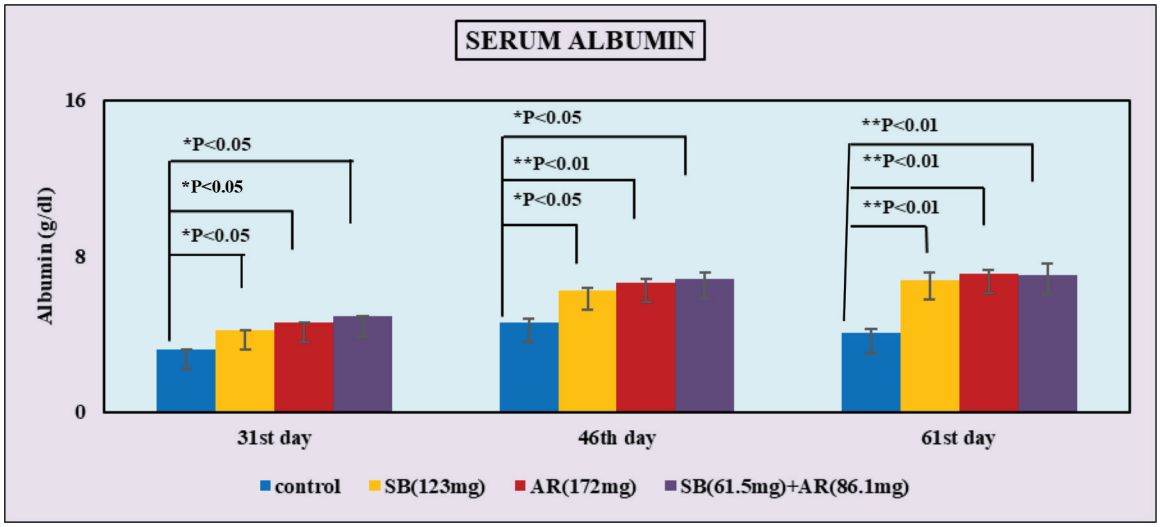


Figure 5. Serum albumin activity on different groups of mice.

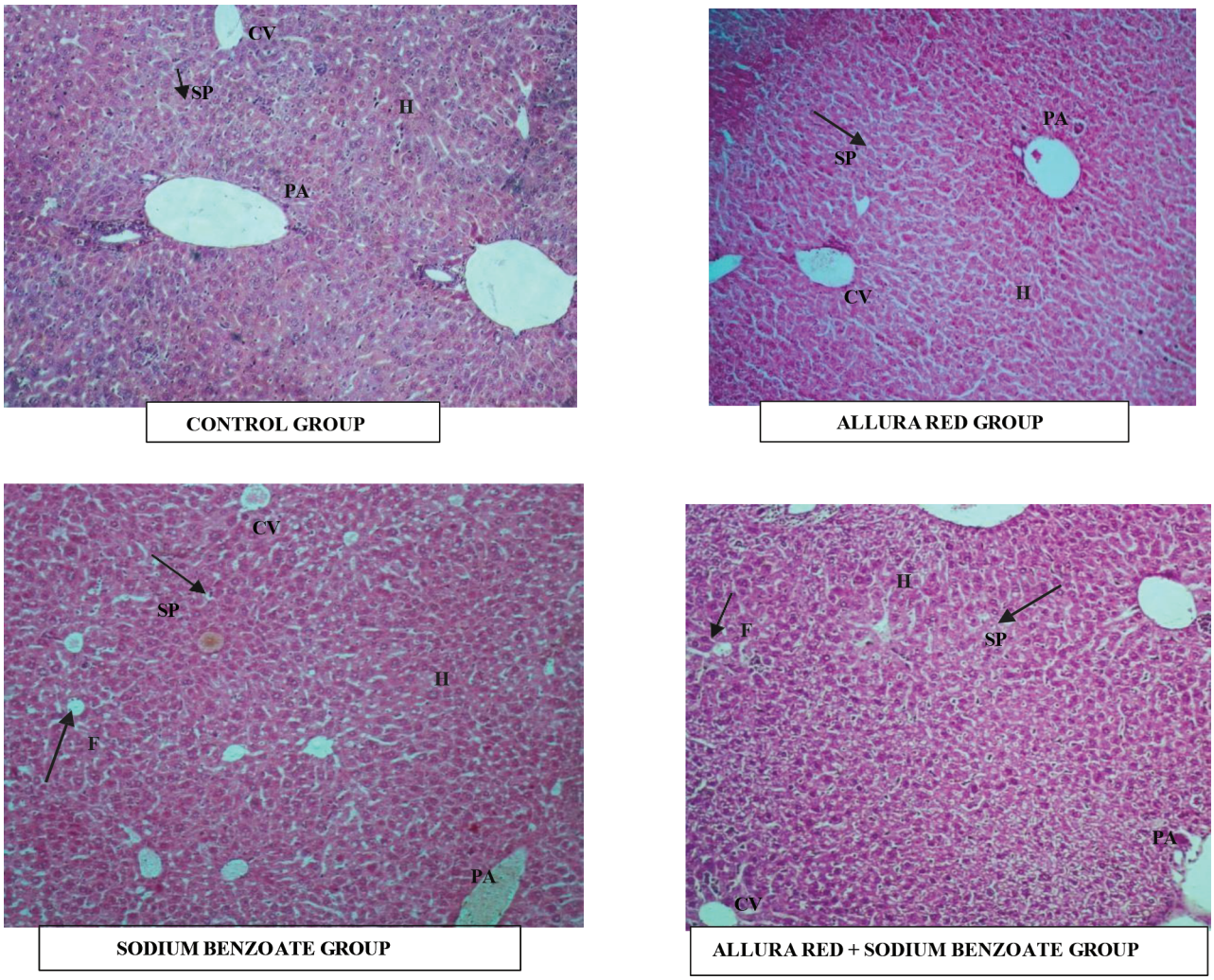


Figure 6. T.S. of the liver section after 30 days' treatment (H and E x100).

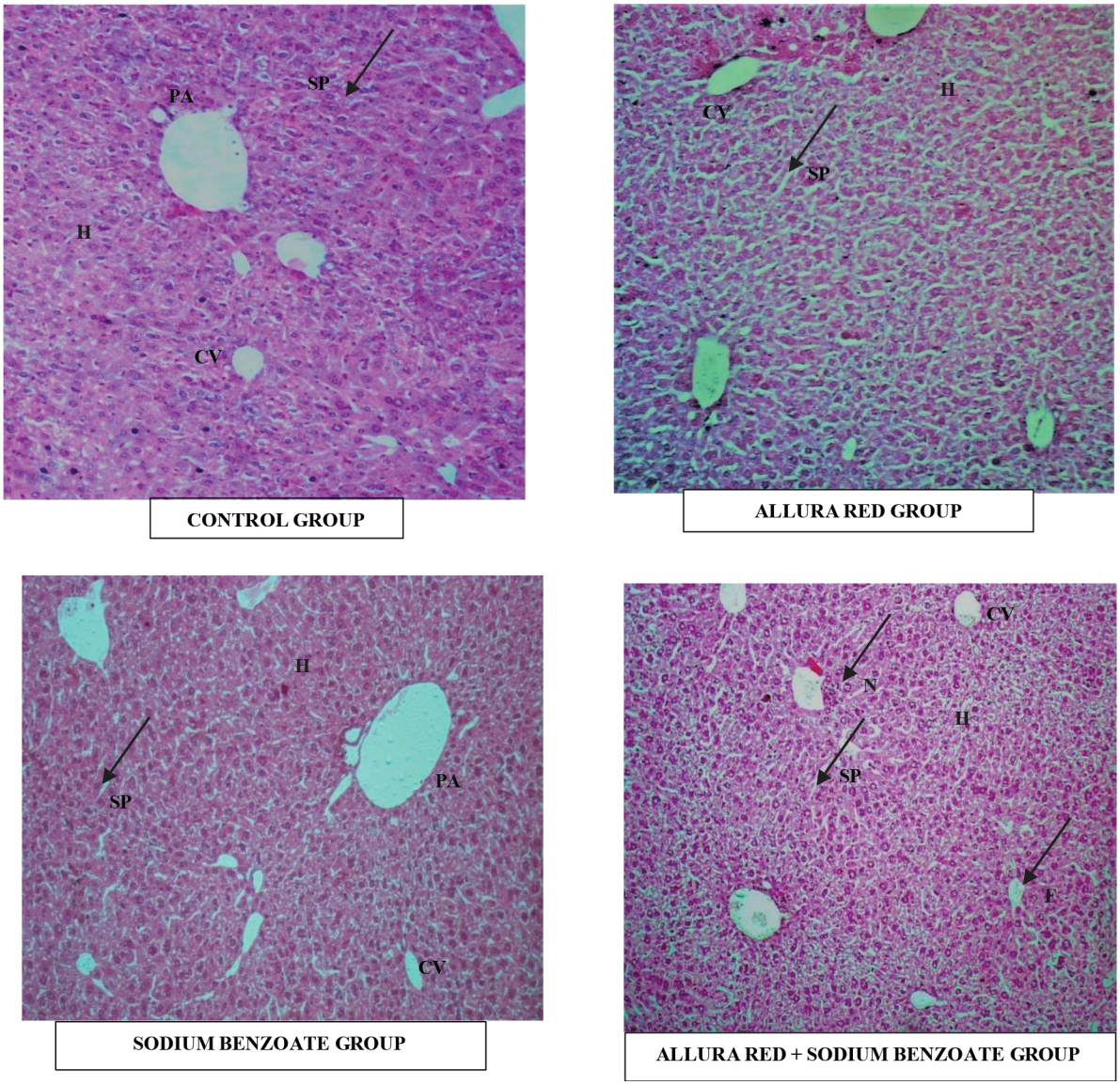


Figure 7. T.S. of the liver section after 45th days' treatment (H andE x100).

Effect of Allura red and sodium benzoate alone and in combination on the liver in mice after 60 days of treatment. The control group showed normal hepatic architecture with the normal CV, PA, H and SP, Allura red, sodium benzoate and a combination showing fatty degeneration of H, V with congestion of CV, necrosis near CV and PA and enlarged sinusoids between the plates of hepatocytes.

There are several uses for food additives, including preservation and colouring. Food colours and preservatives are major ingredients of food in the present lifestyle. They are added to the food to attract consumers and make it more edible[26]. Two of the most used colourant and preservative food additives are AR and SB. Synthetic food colours and preservatives are considered one of the chief suspicions for causing cancer[27]. The present study examines the effect of Allura red (colour), sodium benzoate (preservative) and a combination of AR+SB treatment on the liver parameters of male Swiss Albino mice.

Due to its role as a target organ, a key site of detoxification and the major site of intensive metabolism, the liver is susceptible to a variety of illnesses resulting from exposure to both intrinsic and extrinsic kinds of toxins. According to Guyton and Hall (1996), the liver is critical to metabolism, which preserves the

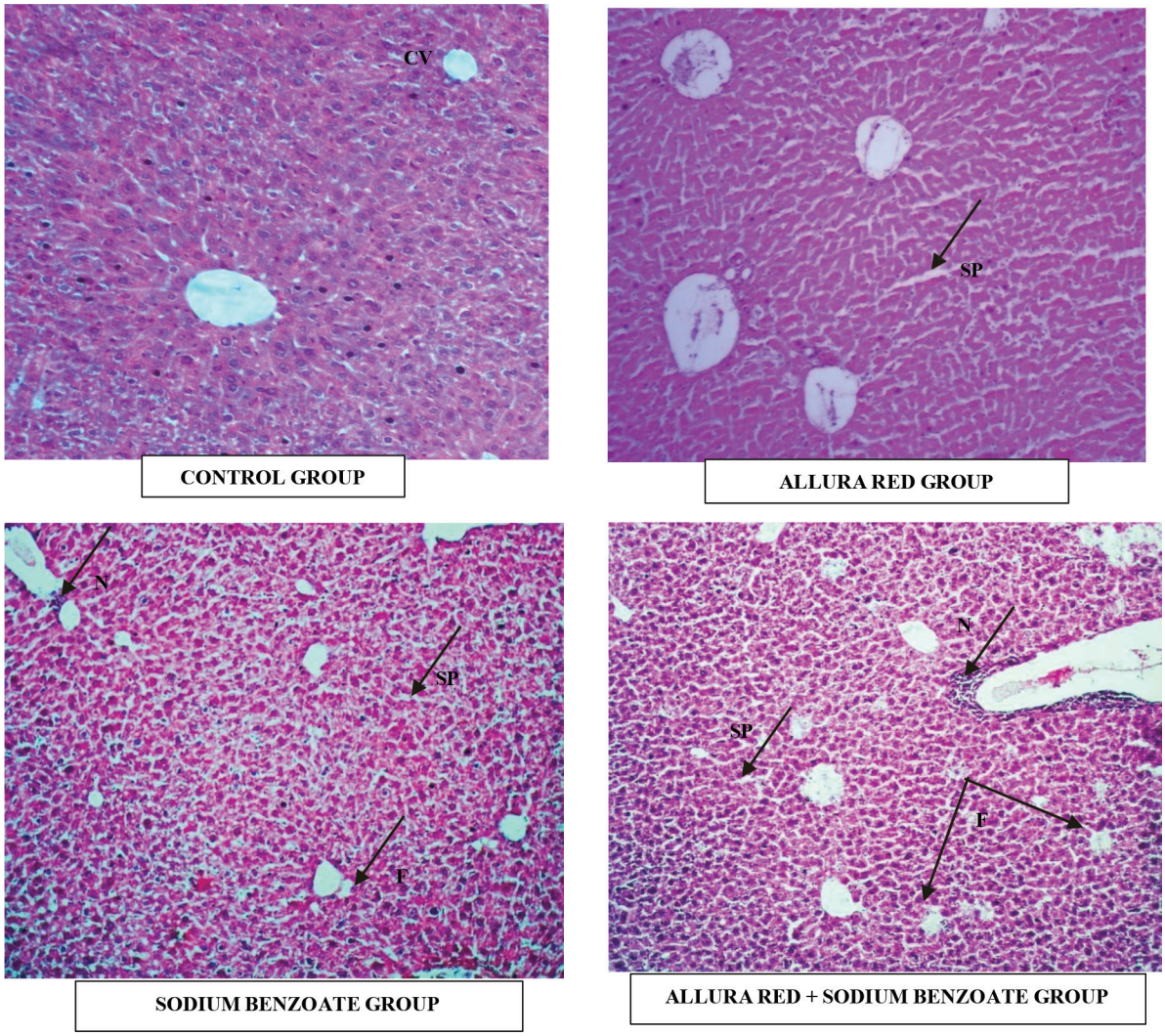


Figure 8. T.S. of the liver section after 60th days' treatment (H and E x100).

body's structural stability and energy levels. The liver is essential for both the biotransformation of xenobiotics and the metabolism of nutrients, which lowers toxicity by changing a hazardous molecule into a less dangerous form. The therapeutic significance of liver enzymes lies in the fact that their efficacy is contingent upon the degree of cellular injury causing their discharge into peripheral fluids and ultimately into the bloodstream.

The liver is crucial to the removal of dangerous substances from blood. When a person is subjected to medication toxicity or poisoning, their hepatocytes may become damaged or die and release enzymes into the bloodstream[28-31].

In the present study, the administration of AR, SB and AR+SB shows marked variations in the liver parameters. The data showed a noticeable increase in ALT, AST, ALP, total protein and albumin levels after 30, 45 and 60 days of all dose treatment as compared to the control group. The amount of biochemical markers released into the bloodstream is used to determine the extent of tissue injury[32].

The measurement of AST and ALT in serum plays a major role in evaluating liver disease. Enzymes that leak into the bloodstream due to damage to the liver's membranes can subsequently be detected in the serum. Elevated levels of AST and ALT in the serum are typically a sign of liver disease. The increased activity of these enzymes indicates a damaged state of the hepatocyte. It also suggests that there is hepatocyte injury or necrosis, as well as increased permeability.

This suggests that a substantial amount of these enzymes have leaked from liver tissue into bodily fluids,

particularly serum and that this high leakage reflects the degree of damage to body tissues, particularly the liver[33]. Studies have demonstrated the toxic effects of sodium benzoate on liver tissue, where it causes the accumulation of cell debris that triggers inflammatory cell responses to injuries. Long-term exposure to benzoate can also cause oxidative damage to the liver tissue, which affects the histological texture of the organ. Several studies have also shown that sodium benzoate metabolises in the liver and conjugates with glycine to form hippuric acid, which is then converted into liver tissue. Glycine acts as a detoxing agent. The histological results of liver and brain tissues show alternation in their structure after treatment with this material[29,34]. Damage to the liver will cause an increase in serum ALP activity due to alkaline phosphatase found in the liver's sinusoidal and canalicular membranes. The considerable increase in the ALP trend provided evidence that sodium benzoate and Allura red have a substantial negative impact on the liver's hepatic capability.

Serum protein levels may be higher due to increased enzyme release from damaged tissues and the need for antibodies to neutralize the dyes. Proteins are the building blocks of all living cells and contain a variety of substances, including hormones, enzymes and antibodies, all of which are essential for an organism to function properly. Protein synthesis may have been activated during inflammation or liver injury, which would explain the rise in total protein. The high albumin levels observed in this investigation could be a sign of sodium benzoate and Allura red harmful effects on hepatic tissues[34,36,39]. The present finding is in agreement with Ifemeje *et al*, who showed an elevated level of ALT, AST and ALP in rats fed with aspartame, sodium benzoate, sodium nitrite and sodium sulfite, Mehdi *et al*, observed an increased level of ALT, AST and ALP in rats when treated with sodium benzoate. Al-Sowayan *et al*, Khan *et al*, and Aziz *et al*, noticed an increased level of ALT, AST, ALP, total protein and albumin in rats fed with sodium benzoate), Alwan *et al,* observed an elevated level of ALT, AST and ALP in rabbits fed with sodium benzoate, Oyewole et al, noticed an increased level of AST and ALT in rats treated with sodium benzoate), Tawfek *et al*, showed an increased level of ALT, AST, ALP, total protein and albumin in rats fed with tartrazine, monosodium glutamate, sunset yellow and sodium benzoate), Al-Shinnawy observed an elevated level of ALT, AST, ALP, total protein and albumin in rats treated with amaranth (azo dye).

The current study found that food colour and preservatives caused fatty degeneration of hepatocytes, vacuolar degeneration, congestion of the CV, necrosis and more SP. These findings are consistent with those of previous research by Bakar and Aktac who noticed degeneration and disarray of the liver section, vacuolation and nucleus losses, pyknotic nuclei by sodium benzoate treatment and by Wu *et al*, who observed hepatocyte damage by azo dye tartrazine. Our findings are in agreement with Saxena and Sharma who discovered that the artificial food dye sunset yellow, metanil yellow and tartrazine exhibited histological alterations in the rat liver. This change consists of vacuolation, infiltration and hepatocyte necrosis. Lymphocytic infiltration around the CV was triggered by the food colouring.

4. Conclusion

In conclusion, the administration of Allura red, sodium benzoate and a combination of AR and SB in mice caused alterations in the liver tissue and biochemical parameters after 30, 45 and 60 days of treatment. The variations signify the toxicity of Allura red, sodium benzoate and combination at the given dose level.

5. Acknowledgments

This work is financially supported by the University Grants Commission (UGC), New Delhi. (UGC ref. no. 951/ (CSIR-UGC net December, 2017).

6. Funding

This work was financially supported by the University Grants Commission (UGC), New Delhi. (UGC Ref. No.:951/ (CSIR-UGC NET DEC. 2017)

7. Reference

1. Pressman P, Clemens R, Hayes W, Reddy C. Food additive safety: A review of toxicological and regulatory issues. SAGE. 2017; 1:1-22. https://doi.org/10.1177/2397847317723572
2. Sharma D, Javed S, Arshilekha, Saxena P, Babbar P, Shukla D, *et al*. Food additives and their effects: A mini-review. Int J Curr Res. 2018.
3. Inetianbor JE, Yakubu JM, Ezeonu SC. Effects of food additives and preservatives on man. Asian J Sci Technol. 2015; 6(02):1118-35.

4. Neelam M, Mishra S. Effects of food additives and preservatives and shelf life of the processed foods. Asian J Sci Technol. 2018; 09(10):8910-12.
5. Helal EGE, Sayed AAR, Mustafa AM, Gamal SM. Adverse effects of two kinds of food additive mixtures (flavor enhancer, food preservative or food coloring agent) on physiological parameters in young male albino rats. Egypt J Hosp Med. 2017; 67(1):344-51. https://doi.org/10.12816/0036646
6. Pandey MR, Upadhyay KS. Food additive. Intech Open. 2012.
7. Gao Y, Li C, Shen J, Yin H, An X, Jin H. Effect of food azo dye tartrazine on learning and memory functions in mice and rats, and the possible mechanisms involved. J Food Sci. 2011; 76. https://doi.org/10.1111/j.1750- 3841.2011.02267.x
8. Saxena B, Sharma S. Food color induced hepatotoxicity in Swiss albino rats, *Rattus norvegicus*. Toxicol Intl. 2015; 22(1). PMid:24748737 PMCid: PMC3989917.
9. Benkhaya S, M'rabet S, EI Harfi A. Classifications, properties, recent synthesis and application of azo dyes. Heliyon. 2020; 6(1):e0327 https://doi.org/10.1016/j.heliyon.2020.e03271 PMid:32042981 PMCid: PMC7002841.
10. Fallico B, Chiappara E, Arena E, Ballistreri G. Assessment of the exposure to Allura red colour from the consumption of red juice based and red soft drinks in Italy. Taylor and Francis. 2011; 28(11):1501-15. https://doi.org/10.1080/19440049.2011.596166 PMid:21854298.
11. Siddiquee S, Shafwanah AMS. Toxicology and analytical methods for the analysis of Allura red (E129) in food and beverage products: A current perspective. Safety Issues in Beverage Production. 2020; 335- 57. https://doi.org/10.1016/B978-0-12-816679-6.00010-3
12. Barciela, P, Perez-Vazquez A, Prieto MA. Azo dyes in the food industry: features, classification, toxicity, alternatives, and regulation. Food and Chemical Toxicology. 2023; 178:113935. https://doi.org/10.1016/j.fct.2023.113935 PMid:37429408.
13. Campos Ventura-Camargo BDE, Aparecida M. Azo dyes: characterization and toxicity- A review. TLIST. 2013; 2(2).
14. Khayyat LI, Essawy AE, Sorour JM, Soffar A. Sunset yellow and Allura red modulate Bcl2 and COX2 expression levels and confer oxidative stress- mediated renal and hepatic toxicity in male rats. Peer J. 2018; 6:5689. https://doi.org/10.7717/peerj.5689 PMid:30280050 PMCid: PMC6166620.
15. Shanmugasundaram P, Bavenro, Rujaswini T. A review on food coloring agents-safe or unsafe? Res J Pharm Technol. 2019; 12(5):2503-5. https://doi.org/10.5958/0974-360X.2019.00421.9
16. Megahed EM, Issa MA, Rahman AA, Mahmoud ME. Effect of using sage on the biological characterization of experimental rats treated with Allura red and brilliant blue pigments. J Food Dairy Sci. 2020; 11(1):9-1. https://doi.org/10.21608/jfds.2020.77964
17. Alsolami MA. Effect of a food additive on certain haematological and biochemical parameters in male albino rat. International Journal of Zoology and Research. 2017;7(2):1-10.
18. Raposa B, Ponusz R, Gerencser G, Budan F, Gyongyi Z, Tibold A, *et al*. Food additives: Sodium benzoate, potassium sorbate, azorubine and tartrazine modify the expression of NFkβ, GADD45α and MAPK8 genes. Physiol Intl. 2016; 103(3):334-43. https://doi.org/10.1556/2060.103.2016.3.6 PMid:28229641.
19. Shahmohammadi M, Javadi M, Nassiri-Asl M. An overview on the effect of sodium benzoate as a preservative in food products. Biotechnol Healthc Sci. 2016; 3(3) https://doi.org/10.17795/bhs-35084
20. Linke BGO, Casagrande TAC, Cardoso LAC. Food additives and their health effects: A review on preservative sodium benzoate. Afr J Biotechnol. 2018; 17(10):306-10. https://doi.org/10.5897/AJB2017.16321
21. Helal EGE, Barayan AW, Abdelaziz MA, EL-Shenawe NSA. Adverse effects of monosodium glutamate, sodium benzoate and chlorophyllins on some physiological parameters in male albino rats. Egypt J Hosp Med. 2019; 74(8):1857-64. https://doi.org/10.21608/ejhm.2019.28865
22. Tawfek NS, Amin HM, Abdalla AA, Fargali SHM. Adverse effects of some food additives in adult male albino rats. Curr Sci Int. 2015; 04(04):525- 37.
23. Radwan EH, Elghazaly MM, Hussein HK, Aziz KKA, Barakat AI. The possible effects of sodium nitrite and sodium benzoate as food additives on the liver in male rats. J Adv Biol. 2020;13. https://doi.org/10.24297/jab.v13i.8717
24. Radwan EH, Elghazaly MM, Hussein HK, Aziz KKA, Barakat A I. Adverse effect of mixture of food additives on some biochemical parameters in male albino rats. J Adv Biol. 2020; 13. https://doi.org/10.24297/jab.v13i.8555
25. Ali MY, Hassan AMS, Mohamed ZA, Ramadan MF. Effect of food colorants and additives on the hematological and histological characteristics of albino rats. Toxicol Environ Health Sci. 2019; 11(2):155-67. https://doi.org/10.1007/s13530-019-0400-x
26. Nair AB, Jacob S. A sample practice guide for dose conversion between animals and human. J Basic Clin Pharma. 2016; 7:27-31. https://doi.org/10.4103/0976-0105.177703 PMid:27057123 PMCid: PMC4804402.
27. Khan IS, Ali MN, Murtaza M. Effect of synthetic red dye orange red and natural red dye alizarin on biochemical and hematological parameters in male Wistar rats. Int J Pharm Sci Drug Res. 2019; 11(5):221-5. https://doi.org/10.25004/IJPSDR.2019.110511

28. Tsuda S, Murakami M, Matsusaka N, Kano K, Taniguchi K, Sasaki YF. DNA damage induced by red food dyes orally administered to pregnant and male mice. Toxicol Sci. 2001; 61(1):92-9. https://doi.org/10.1093/toxsci/61. 1.92 PMid:11294979.
29. Rasgelel PG, Kaymak F. Effect of food preservative natamycin on liver enzyme and total protein in *Mus musculus*. Bulg J Agric Sci. 2013; 19(2):298-302.
30. Mehdi LA, Hasson AM. Estimate levels of the liver enzymes and serotonin immune localization in the brain of rats administrated orally with sodium benzoate. International J Pharm Res. 2020; 12(4):2752-60. https://doi.org/10.31838/ijpr/2020.12.04.380
31. Kadhum SAH. Effect of different dosage from lead acetate administrated on the liver enzymes and histopathological of liver in white male rats. J Pharm Sci Res. 2019; 11(4):1657-61.
32. Sharma UK, Kumar R, Ganguly R, Gupta A, Sharma AK, Pandey AK. Cinnamaldehyde, an active component of cinnamon provides protection against food colour-induced oxidative stress and hepatotoxicity in albino Wistar rats. Vegetos. 2018; 31(2):123-9. https://doi.org/10.5958/2229-4473.2018.00063.0
33. Alwan NA, Kudayer AM, Al-masoudi EA. The toxic effect of oral gavaged of sodium benzoate (SB) on adult male rabbits. Malaysian Journal of Medicine and Health Sciences. 2022; 18(4):64-9.
34. Ifemeje JC, Ezeonyemalu UE, Egbuna C, Olisah MC, Ifemeje MO. Effects of four different food additives on the oxidative stress markers of Wistar albino rats. Int Ann Sci. 2020; 9(1):46-51. https://doi.org/10.21467/ias.9.1.46-51
35. Al-sowayan NS, Sobhy HM, Omaima, Hassan MM, Al-sallali RM, Hanan, Sultan A. Effect of sodium benzoate on DNA breakage and antioxidant enzymes in male rats. Egypt. J Chem Environ Health. 2021; 7(1):1-9. https://doi.org/10.21608/ejceh.2021.233150
36. Khan IS, Dar KB, Ganie SA, Ali MN. Toxicological impact of sodium benzoate on inflammatory cytokines, oxidative stress and biochemical markers in male Wistar rats. Drug Chem Toxicol. 2020; 45(3):1345-54. https://doi.org/10.1080/01480545.2020.1825472 PMid:33003957.
37. Aziz IISA, Zabut BMH. Blood indices of sodium benzoate administrated albino rats: Effect of olive oil and/or time-dependent recovery. Egypt J Biol. 2012; 14:50-6.
38. Oyewole OI, Dere FA, Okoro OE. Sodium benzoate mediated hepatorenal toxicity in Wistar rat: Modulatory effects of *Azadirachta indica* (neem) leaf. European J Med Plants. 2012; 2(1):11-8. https://doi.org/10.9734/EJMP/2012/619
39. Al-Shinnawy MS. Physiological effect of a food additive on some haematological and biochemical parameters of male albino rats. Egypt. Acad. J Bio Sci. 2009; 2(1):143-51. https://doi.org/10.21608/eajbsa.2009.15511
40. Amin KA, Hameid HA, Elsttar AHA. Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food Chem Toxicol. 2010; 48:2994-9. https://doi.org/10.1016/j.fct.2010.07.039 PMid:20678534.
41. Bakar E, Aktac T. Effect of sodium benzoate and citric acid on serum liver and kidney tissue total sialic acid level an ultrastructure study. J Appl Biol Sci. 2014; 8(2):9-15.
42. Wu L, Xu Y, Lv X, Chang X, Ma X, Tian X, Shi X, Li X, Kong X. Impacts of an azo dye food dye tartrazine uptake on intestinal barrier, oxidative stress, inflammatory response and intestinal microbiome in crucian carp (*Carassius auratus*). Ecotoxicol Environ Saf. 2021; 223:112551. https://doi.org/10.1016/j.ecoenv.2021.112551 PMid:34358931.