ORIGINAL ARTICLE

Bali Medical Journal (*Bali MedJ*) 2022, Volume 11, Number 3: 1755-1759
P-ISSN.2089-1180, E-ISSN: 2302-2914

Effect of tartrazine on blood urea nitrogen, creatinine levels, and renal tubular necrosis in adult male Wistar rats (Rattus norvegicus): an experimental study

Mulyati Sri Rahayu[1*], Sri Wahyuni[2], Idha Fitriani[3], Habib Budiman Agung[3]

[1]Department of Anatomical Pathology, Faculty of Medicine, Universitas Malikussaleh, Aceh, Indonesia;
[2]Department of Biochemistry, Faculty of Medicine, Universitas Malikussaleh, Aceh, Indonesia;
[3]Undergraduate student, Faculty of Medicine, Universitas Malikussaleh, Aceh, Indonesia;

*Corresponding author:
Mulyati Sri Rahayu;
Department of Anatomical Pathology, Faculty of Medicine, Universitas Malikussaleh, Aceh, Indonesia;
mulyati.srirahayu@unimal.ac.id

Received: 2022-08-07
Accepted: 2022-10-22
Published: 2022-11-28

ABSTRACT

Introduction: Tartrazine is still one of the most widely used coloring agents in the pharmaceutical, cosmetic, and food industries; various hazardous consequences have been identified in rodents and humans, including impaired kidney function. This current study aimed to evaluate the effect of tartrazine on the biochemical parameters and structures of the kidney.
Methods: Twenty-four adult male Wistar rats were divided into four groups of 6 each. The experimental animals received tartrazine orally at a dose of 3.75, 7.5, and 15 mg/kg body weight along with a normal diet. The control group received only food and drinking water. The study was carried out for 21 days. At the end of the experiment, biochemical (blood urea nitrogen and serum creatinine) and histopathological examinations were performed on animal kidney tissues.
Results: Our findings revealed a significant increase in urea and creatinine levels in the serum of tartrazine-treated rats compared to controls ($p < 0.05$). The kidney section of rats treated with tartrazine showed lumen compression of tubular cells and loss of integrity of the renal tubule membrane. Tartrazine was associated with increases in the percentage of renal tubular necrosis in a dose-dependent manner compared to the control group ($p < 0.05$).
Conclusions: The current study concluded that oral administration of tartrazine affected the kidney by increasing urea nitrogen, creatinine levels, and the percentage of renal tubular necrosis in male Wistar rats. The results showed that tartrazine intake could cause adverse kidney health effects.

Keywords: tartrazine, urea, creatinine, rat kidney, necrosis.
Cite This Article: Rahayu, M.S., Wahyuni, S., Fitriani, I., Agung, H.B. 2022. Effect of tartrazine on blood urea nitrogen, creatinine levels, and renal tubular necrosis in adult male Wistar rats (Rattus norvegicus): an experimental study. *Bali Medical Journal* 11(3): 1755-1759. DOI: 10.15562/bmj.v11i3.3623

INTRODUCTION

Tartrazine, a widely used artificial dye chemically derived from coal tar paint, is used in the production of a wide range of food products, pharmaceutical industries, and cosmetics.[1] It can also be found in dietary supplements and medical capsules.[2–4] Synthetic dyes have many advantages over natural dyes, including chemical resistance to oxidation and changes in pH and temperature, color intensity, and uniformity, and are cheaper.[5,6]

Tartrazine is represented by the symbol E102 since the letter E denotes the permission to use the additive in all countries of the European Union and denotes its safety and the agreed concentration that does not cause adverse effects.[3,6,7] The safety of these tar dyes has not been proven, despite strict guidelines on acceptable daily intake (ADI).[5] ADI is defined as an estimate of the amount of food or drinking water additive that can be consumed safely daily for a lifetime without causing adverse health effects. The ADI of TZ for humans is 0 to 7.5 mg/kg bw.[8–10] In 2016, FAO/WHO (JECFA) and EFSA re-evaluated the ADI of 0 to 10 mg/kg bw/day.[10] According to EFSA and others, the lethal dose for Wistar rats is greater than 2000 mg/kg bw.[11,12]

In several developing countries, food coloring was used in excess of ADI on multiple occasions, which would cause serious health complications in humans.[13] Tartrazine is one of the 11 artificial organic dyes allowed in Indonesia.[14] Due to the high consumption of instant noodles and soft drinks, the estimates of dietary exposure in Indonesia (0.21–0.64 mg/kg bw/day) were ten times higher than in other countries.[10]

Although many studies have been conducted in laboratory animals to find toxic effects of tartrazine in the current study,[15–18] significant progress is still required to understand the effects in vivo on the kidney. Therefore, this study aims to evaluate the effect of oral administration of tartrazine in white rats (Rattus norvegicus) on urea nitrogen level, creatinine level, and histopathological renal tubular necrosis.

MATERIALS AND METHODS

The tartrazine study was carried out for 21 days, in which male adult Wistar rats (Rattus norvegicus) received food and substances daily ad libitum. Before the experiment started, the rats were acclimatized for one week under controlled conditions (12 hours of light / dark cycle)

in the Animal Laboratory of Syiah Kuala University. They were allowed free access to basic food and water.

Experimental Design

Twenty-four (24) adult male Wistar rats (Rattus norvegicus) weighing 150-200 grams were used in this study. The animals were randomly divided into four groups; each had six animals per cage. Tartrazine was used in the ADI range recommended by JECFA and the Indonesian FDA (BPOM) (3,75; 7,5; and 15 mg/kg-b.w/day). The dose was converted according to the animal equivalent dose using the formula of Nair and Jacob.[19] Treatment was; **(I)** normal control group (C) received only food and drinking water for the entire period of the experiment; **(II)** group 1 received 3.75 mg/kg bw; **(III)** group 2 received 7.5 mg/kg bw; **(IV)** group 3 received 15 mg/kg bw.

Chemical

The present study purchased tartrazine from Sayona Colors PVT LTD (LOT NO.SCLP/152).

Blood and organ collection

After 21 days of treatment, the control and experimental animals were sacrificed by cervical decapitation under anesthesia. Blood samples were taken from the orbital sinus; serum was separated and used for biochemical analysis. The kidney specimens were quickly removed and fixed in an appropriate fixative for histological studies.

Biochemical analysis

Blood urea nitrogen and serum creatinine were measured using an automatic hematology analyzer.

Histological Examination

Kidney samples were collected and fixed in 10% phosphate-buffered formalin for 24 h, routinely processed, and stained with hematoxylin and eosin (H&E). The sample was examined using an Olympus CX33 microscope. Photomicrographs were taken with Cellsen imaging software (Olympus Corporation, Japan). All samples were evaluated for histological changes, including the percentage of renal tubular necrosis.

Statistical analysis

The normal distribution of the values for each biochemical parameter (blood urea nitrogen and creatinine) was verified using the Shapiro-Wilk test. All data obtained by this experiment are presented in tables or figures as mean ± S.D. The statistical significance of the differences between the control and experimental groups was evaluated using a one-way analysis of variance (ANOVA) using computer statistical software. Significant mean differences between groups (normal control, 1, 2, 3, 4) were further examined using the LSD test. A $p<0.05$ was considered statistically significant.

RESULTS

Biochemical results

Table 1 shows the mean values and standard deviation of the kidney function indices (serum urea nitrogen and creatinine) determined by biochemical analysis of blood. Treatment with tartrazine significantly increased urea and creatinine levels ($p = 0.000$). Biochemical blood analysis revealed significant variations in urea and creatinine levels depending on tartrazine dose.

All rats that received tartrazine showed a highly significant increase in urea concentration compared to control rats. Significant increases values ($p < 0.05$) were registered for group 3 (47.33 ± 8), followed by group 2 (41.17 ± 5.9), 1 (34.67 ± 4.8) compared to the control group (25.33 ± 4.1), respectively. Furthermore, there was a significant increase ($p < 0.05$) in the groups fed a higher dose of tartrazine (47.33 ± 8) compared to group 1 (34.67 ± 4.8).

Regarding creatinine, tartrazine significantly increased serum creatinine ($p < 0.01$) in a dose-dependent manner. Serum creatinine concentration (mg/dl) was elevated in values of (0.93 ± 0.03), (1.01 ± 0.06), and (1.15 ± 0.06) after daily oral administration of tartrazine compared to control (0.78 ± 0.03). Additionally, a significant value was observed in group

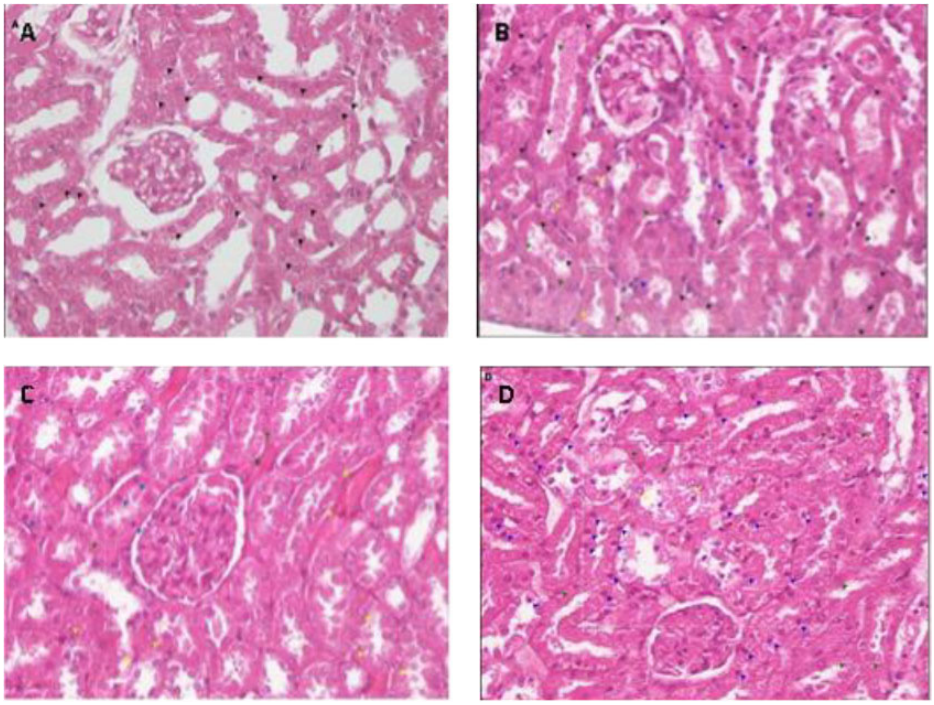

Figure 1. Light micrographs of kidney sections from control and tartrazine-treated rats stained with H&E (400X). A: Control rats showing normal rat kidney with normal tubular brush-borders; Kidney section from rat treated with tartrazine; Group 1 (B), Group 2 (C), and Group 3 (D): showing compression of the tubular lumen, loss of renal tubules membrane integrity, and increased tubular epithelial cell necrosis. Note: black arrows (normal cells), blue arrows (pyknotic cells), yellow arrows (karyorrhexis cells), and green arrows (karyolysis).

Table 1. Blood urea nitrogen and creatinine levels in control and the treatment groups.

Parameters	Control	Group 1	Group 2	Group 3
Urea (mg/dl)	25.33 ± 4.1	34.67 ± 4.8[ab]	41.17 ± 5.9[a]	47.33 ± 8[ac]
Creatinine (mg/dl)	0.78 ± 0.03	0.93 ± 0.03[ab]	1.01 ± 0.06[a]	1.15 ± 0.06[ac]

Notes:
Values are mean ± standard deviation (n = 6). a: significant difference from the control group. While, within columns, means followed by the different letters (b and c) are significantly different between treatments according to the Post Hoc Test (LSD) ($p < 0.05$).

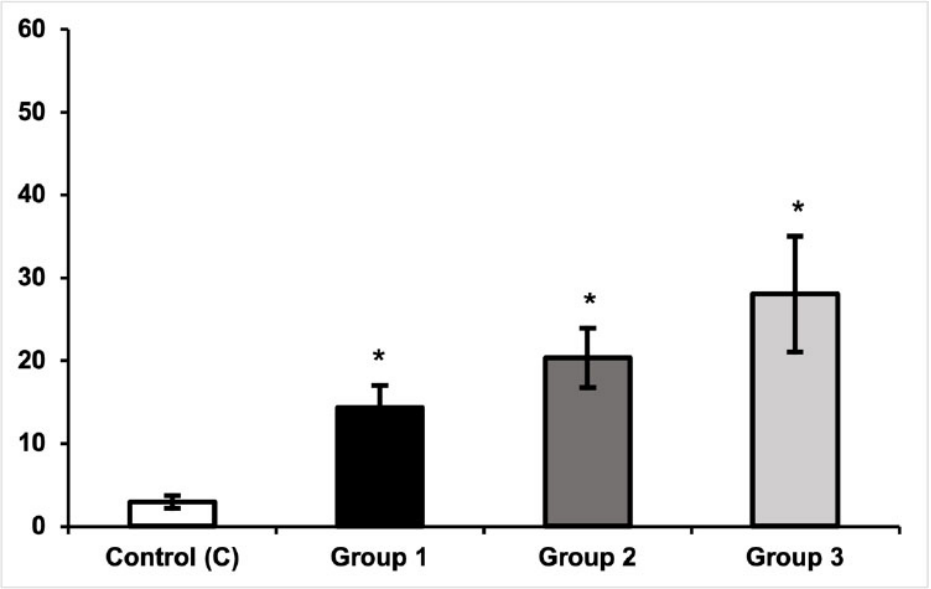

Figure 2. Percentage of renal tubular necrosis. Data represented as mean ± SD (n = 6). *p-value for Post Hoc Test (LSD) test between control and treated groups (p= 0.000). Values are statistically significant at $p < 0.05$.

3 (1.15 ± 0.06). Furthermore, when the groups were compared using the Pos Hoc (LSD) test, significantly higher means were seen between group 1 (0.93 ± 0.03) and group 3 (1.15 ± 0.06).

Histological result

The photomicrographs showed changes in the histopathological structure of tubular epithelial cells with nuclear changes, including pyknotic, karyorrhexis, and karyolysis (Figure 1).

Macroscopic examination of the kidneys of the control group showed a normal appearance. Histological changes in kidney structure were observed in the animal group treated with tartrazine compared to the controls. Control rats showed normal rat kidneys with normal tubular brush borders. The kidney section of rats treated with tartrazine showed tubular cell lumen compression, loss of renal tubule membrane integrity, and increased tubular epithelial cell necrosis in a dose-dependent manner.

The average percentage of renal tubular cell necrosis in group 1, group 2, and group 3 was 14.29 ± 2.76%, 20.36 ± 3.58%, and 28.03 ± 6.99%, respectively, compared to the control group (2.95 ± 0.77%) (Figure 2). The one-way ANOVA test revealed that the percentage of renal tubular necrosis differed significantly between the groups. The LSD test showed a statistically significant difference in the percentage of renal tubular necrosis in the control group and all treatment groups (p = 0.000).

DISCUSSION

The present study shows the effect of tartrazine (azo dye) on kidney function revealed by biochemical tests and histological changes. We examine the effect of tartrazine treatment on the kidneys of adult male rats given the ADI dose of tartrazine for 21 days.

The normal control group had a high urea value (25.33 mg/dl) compared to its baseline values. Rat blood urea nitrogen levels are generally in the 15 to 22 mg/dl range. However, the creatinine level remains normal (0.78 mg/dl), within the range of 0.4 to 0.8 mg/dl.[20] Chemically induced increases in BUN and/or serum creatinine, on the other hand, could be due to dehydration, hypovolemia, muscle injury, or protein catabolism rather than renal damage.[21,22] This could be attributed to rats dehydrated during the experiment because they only had access to water in one location, resulting in rats not drinking sufficiently. Tartrazine consumption may also have contributed to low food and water intake. It was hypothesized that the addition of synthetic colorants or flavors to the diet would inhibit digestion in a particular way.[18]

To investigate kidney function, we measured serum urea and creatinine concentration in tartrazine-treated rats and found an elevated value, as predicted. The most common measurement of kidney function is urea, or blood urea nitrogen (BUN), and serum creatinine. Compared to the control group, serum levels of urea and creatinine are significantly elevated in the tartrazine-treated group. Amin et al. observed a similar finding in rats that ingested low (8 and 15 mg/kg/day) or high doses (100 and 500 mg/kg/day) of tartrazine for 30 days.[15] These results also agree with Tawfek et al., who found a significant increase in serum nitrogen and creatinine in rats after consuming different food color additives, including tartrazine.[17] Ali et al. also showed that tartrazine induces a significantly increased creatinine and urea level.[23] Additionally, this result is in agreement with data reported by Khayyat et al. They revealed that tartrazine 7.5 mg/kg bw significantly increases serum creatinine and urea levels in rats. Furthermore, elevated serum creatinine and urea levels were also found in male rats administered 7.5 mg/kg/day and 75 mg/kg/day for seven weeks in a study by Usman et al.[24] Recently, Shakoor et al. reported that tartrazine consumption induced significant elevations in urea and creatinine levels in rats given a lower and higher dose of ADI (9.6 and 96 mg/kg bw) daily for 15, 30, and 45 days.

The renal clearance of endogenous substances, which also indicates renal

dysfunction, is determined by measuring urea or creatinine in plasma. The BUN level measures the kidney's ability to filter blood; it does not increase significantly until kidney function is reduced by 60 to 75%.[25,26] Elevated levels of blood urea nitrogen and/or plasma creatinine frequently indicate a decrease in glomerular filtration rate. BUN concentration is inversely proportional to GFR; elevated levels can suggest kidney injury.[27,28] At the concentration tested in this present study, tartrazine appears to induce renal dysfunction.

In the current work, histological findings demonstrated that the administration of tartrazine to rats resulted in distortion and degeneration of the kidney architecture and necrosis of the renal tubule membranes. Numerous ultrastructural alterations, such as irregular or pyknotic nuclei in the epithelium of the renal tubules, were observed in the kidneys of rats treated with tartrazine. These findings are consistent with Khayyat et al., who indicated that tartrazine administration of 7.5 mg/kg bw for 30 days alters the histological structure of the kidneys in experimental animals.[29] Furthermore, these histopathological alterations are consistent with El-sakhawy et al. They discovered that the proximal convoluted tubule (PCT) and the distal convoluted tubule showed poorly defined cell boundaries in renal tissue obtained from adult male albino rats treated with doses of 7.5, 15, and 100 mg/kg bw. Some PCTs demonstrated pyknotic nuclei, a poorly defined brush border, and epithelium loss.[30] These morphological changes may be due to the proximal tubule being the most common site of toxicant-induced renal dysfunction.[22,31] Furthermore, proximal tubules have higher cytochrome P-450, which detoxifies or activates toxicants and is often the site of adverse effects.[32]

Cells in the kidney tubule can be exposed to concentrations of a toxic substance that are many times higher than the plasma concentration of that toxin.[33] In a study conducted by Balta et al. tubular dilatation, tubular degeneration, glomerular capillary dilatation, intracapillary sclerosis, and glomerulus atrophy were observed. In addition to altering kidney parameters, tartrazine can induce oxidative stress by forming free radicals, leading to a more dangerous effect at higher doses.[34] More than one test result would likely be required to draw a definitive conclusion about renal injury, typically in conjunction with a histopathological evaluation.[27] An increase in blood urea and creatinine was confirmed in this study with a kidney lesion.

Necrosis is the end stage of the cell or tissue death process in living organisms. The nuclei of dead cells can be seen to be smaller, and chromatin and reticular fibers fold more. The nucleus becomes denser (pyknotic) and can disintegrate into segments (karyorrhexis) and then becomes eosinophilic (karyolysis). Suppose that a chemical is actively secreted from blood into urine; in that case, the chemical accumulates first in the renal tubules. If this chemical is reabsorbed from the urine, it will pass through tubular epithelial cells at high concentrations. The concentration process causes these toxic substances to accumulate in the kidneys and causes a narrowing of the tubular lumen.[21,22,26] Tubular epithelial cells are primarily responsible for the structural and functional recovery of the nephron after injury by replacing dead and detached cells through dedifferentiation, proliferation, migration, and redifferentiation.[26]

CONCLUSIONS

Administration of the ADI dose of tartrazine showed a significant effect on urea nitrogen, creatinine levels, and the percentage of renal tubular necrosis in the Wistar strain of white male rats (Rattus norvegicus), indicating renal dysfunction. Therefore, it is highly recommended to limit tartrazine consumption, especially in foods consumed by children, to maintain good health.

DISCLOSURE

Conflict of Interest

The author declares that there are no conflicts of interest related to the material presented in this article.

Ethics Consideration

Ethics approval has been obtained from the Lembaga Peneliti Muda Kesehatan Aceh-Indonesia Health Research Ethics Committee (number: 13/KOMET/LPMKA/2017).

Funding

None.

Authors Contribution

All authors contributed equally to the study, from the conceptual framework, data collection, and data analysis, to reporting the study results through publication.

REFERENCES

1. El-Borm H, Badawy G, Hassab El-Nabi S, El-Sherif W, Atallah M. Toxicity of sunset yellow fcf and tartrazine dyes on dna and cell cycle of liver and kidneys of the chick embryo: the alleviative effects of curcumin. Egypt J Zool [Internet]. 2020;74(74):43–55. Available from: http://dx.doi.org/10.21608/ejz.2020.42218.1040
2. Kamal AA, Fawzia SES. Toxicological and safety assessment of tartrazine as a synthetic food additive on health biomarkers: A review. African J Biotechnol [Internet]. 2018;17(6):139–49. Available from: http://dx.doi.org/10.5897/ajb2017.16300
3. Thurmond TS. U. S. Food and Drug Administration's (FDA) Safety Assessment of Food Ingredients [Internet]. ACS Symposium Series. American Chemical Society; 2014. p. 91–5. Available from: http://dx.doi.org/10.1021/bk-2014-1162.ch009
4. Silva MM, Reboredo FH, Lidon FC. Food Colour Additives: A Synoptical Overview on Their Chemical Properties, Applications in Food Products, and Health Side Effects. Foods (Basel, Switzerland) [Internet]. 2022 Jan 28;11(3):379. Available from: https://pubmed.ncbi.nlm.nih.gov/35159529
5. El Golli N, Bini-Dhouib I, Jrad A, Boudali I, Nasri B, Belhadjhmida N, et al. Toxicity Induced after Subchronic Administration of the Synthetic Food Dye Tartrazine in Adult Rats, Role of Oxidative Stress. Recent Adv Biol Med [Internet]. 2016;02:20. Available from: http://dx.doi.org/10.18639/rabm.2016.02.284474
6. Amchova P, Kotolova H, Ruda-Kucerova J. Health safety issues of synthetic food colorants. Regul Toxicol Pharmacol [Internet]. 2015;73(3):914–22. Available from: http://dx.doi.org/10.1016/j.yrtph.2015.09.026
7. Steele EA, Breen C, Campbell E, Martin R. Food Regulations and Enforcement in the USA [Internet]. Reference Module in Food Science. Elsevier; 2016. Available from: http://dx.doi.org/10.1016/b978-0-08-100596-5.21031-7
8. Scientific Opinion on the appropriateness of the food azo-colours Tartrazine (E 102), Sunset Yellow FCF (E 110), Carmoisine (E 122), Amaranth (E 123), Ponceau 4R (E 124), Allura Red AC (E 129), Brilliant Black BN (E 151), Brown FK (E 154), Brown HT (E 155. EFSA J

[Internet]. 2010;8(10):1778. Available from: http://dx.doi.org/10.2903/j.efsa.2010.1778
9. Scientific Opinion on the re-evaluation Tartrazine (E 102). EFSA J [Internet]. 2009;7(11):1331. Available from: http://dx.doi.org/10.2903/j.efsa.2009.1331
10. Compendium of Food Additive Specifications-Joint FAO/WHO Expert Committee on Food Additives (JECFA) [Internet]. FAO and WHO; 2021. Available from: http://dx.doi.org/10.4060/cb3310en
11. NLM PubChem CID Index [Internet]. Vitamin D Handbook. John Wiley & Sons, Inc.; p. 239–44. Available from: http://dx.doi.org/10.1002/9780470238165.indsp1
12. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, et al. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res Toxicol Environ Mutagen [Internet]. 2002;519(1–2):103–19. Available from: http://dx.doi.org/10.1016/s1383-5718(02)00128-6
13. Rao P, Bhat R V, Sudershan R V, Prasanna Krishna T. Consumption of synthetic food colours during festivals in Hyderabad, India. Br Food J [Internet]. 2005;107(5):276–84. Available from: http://dx.doi.org/10.1108/00070700510596875
14. Gondokesumo ME, Amir N. The Role of Government Supervision and the Food and Drug Supervisory Agency (BPOM) in the Circulation of Counterfeit Drugs in the State of Indonesia (Judging from Law Number 36 Year 2009 and Regulation of the Head of the Drug and Food Management Agency) [Internet]. 2021;91–107. Available from: http://dx.doi.org/10.30649/ph.v21i2.16
15. Amin KA, Abdel Hameid H, Abd Elsttar AH. 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 [Internet]. 2010;48(10):2994–9. Available from: http://dx.doi.org/10.1016/j.fct.2010.07.039
16. Singh P. 90 Day Repeat DOSE Oral Toxicity Study of GM Derived Cotton Seed In Wistar Rats. Am J PharmTech Res [Internet]. 2019;9(3):36–73. Available from: http://dx.doi.org/10.46624/ajptr.2019.v9.i3.003
17. Tawfek NS, Amin HM, Abdalla AA, Fargali SHM. Adverse Effects of Some Food Additives in Adult Male Albino Rats. Current Science International. 2015;04(04):525–37.
18. El-Wahab HMFA, Moram GSED. Toxic effects of some synthetic food colorants and/or flavor additives on male rats. Toxicol Ind Health [Internet]. 2012;29(2):224–32. Available from: http://dx.doi.org/10.1177/0748233711433935
19. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J basic Clin Pharm [Internet]. 2016 Mar;7(2):27–31. Available from: https://pubmed.ncbi.nlm.nih.gov/27057123
20. Bullock G, Bunton TE. Foreword [Internet]. The Laboratory Rat. Elsevier; 2000. p. xv. Available from: http://dx.doi.org/10.1016/b978-012426400-7.50038-8
21. Timbrell JA. Principles of Biochemical Toxicology [Internet]. CRC Press; 2008. Available from: http://dx.doi.org/10.3109/9781420007084
22. Tarloff JB, Wallace AD. Biochemical Mechanisms of Renal Toxicity [Internet]. Molecular and Biochemical Toxicology. John Wiley & Sons, Inc.; p. 693–724. Available from: http://dx.doi.org/10.1002/9780470285251.ch29
23. Saeid Abdelgayed S. Histopathological and immunohistochemical studies of long term administration of nutmeg on parotid salivary glands in albino rats [Internet]. Morressier; 2016. Available from: http://dx.doi.org/10.26226/morressier.578f37fdd462b8028d88fb56
24. Usman JN, Muhammad GA. Sub-acute toxicity study on tartrazine in male albino rats. Dutse J Pure Appl Sci [Internet]. 2022;8(1b):97–105. Available from: http://dx.doi.org/10.4314/dujopas.v8i1b.12
25. Gad SC. Rodent Models for Toxicity Testing and Biomarkers [Internet]. Biomarkers in Toxicology. Elsevier; 2019. p. 7–73. Available from: http://dx.doi.org/10.1016/b978-0-12-814655-2.00002-5
26. Schnellmann RG. Toxic response of the kidney. In: Klaassen CD, Watkins III JB, editors. Casarett & Doulls Essentials of Toxicology. McGraw-Hill Education ; 2015. p. 209–30.
27. Robinson L. A Practical Guide to Toxicology and Human Health Risk Assessment [Internet]. John Wiley & Sons, Inc.; 2018. Available from: http://dx.doi.org/10.1002/9781118881880
28. Nabila M, Nawel M, Omar A, Soraya AT, Chahinaize Z, Omar K, et al. A thirteen week ad libitum administration toxicity study of Tartrazine in Swiss mice. African J Biotechnol [Internet]. 2013;12(28):4519–29. Available from: http://dx.doi.org/10.5897/ajb2013.12125
29. Khayyat L, Essawy A, Sorour J, Soffar A. Tartrazine induces structural and functional aberrations and genotoxic effects in vivo. PeerJ [Internet]. 2017 Feb 23;5:e3041–e3041. Available from: https://pubmed.ncbi.nlm.nih.gov/28243541
30. El-sakhawy MA, Mohamed DW, Ahmed YH. Histological and immunohistochemical evaluation of the effect of tartrazine on the cerebellum, submandibular glands, and kidneys of adult male albino rats. Environ Sci Pollut Res [Internet]. 2019;26(10):9574–84. Available from: http://dx.doi.org/10.1007/s11356-019-04399-5
31. Cottrell S. Book Reviews : Fundamental Toxicology for Chemists Editors: John H Duffus and Howard GJ Worth The Royal Society of Chemistry 1996, 327pp. £29.50. Hum & Exp Toxicol [Internet]. 1997;16(4):237. Available from: http://dx.doi.org/10.1177/096032719701600420
32. Lee BM, Kacew S. Lu's Basic Toxicology [Internet]. CRC Press; 2012. Available from: http://dx.doi.org/10.3109/9781841849546
33. Stine KE, Brown TM. Principles of Toxicology [Internet]. CRC Press; 2015. Available from: http://dx.doi.org/10.1201/b18343
34. Balta I, Sevastre B, Mireşan V, Taulescu M, Raducu C, Longodor AL, et al. Protective effect of blackthorn fruits (Prunus spinosa) against tartrazine toxicity development in albino Wistar rats. BMC Chem [Internet]. 2019 Aug 9;13(1):104. Available from: https://pubmed.ncbi.nlm.nih.gov/31417987