Send Orders for Reprints to reprints@benthamscience.net

Applied Clinical Research, Clinical Trials & Regulatory Affairs, **2019**, *7*, 1-6

MINI-REVIEW

Genotoxic Impurities in Ranitidine Containing Products: An Overview

Shailesh Bhosale[1], Kamal Kant[2], Divya Goyal[1] and Anoop Kumar[1,*]

[1]*Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research, Raebareli (NIPER-R)-226002, Lucknow (U.P.)-India;* [2]*Independent Researcher, Hamirpur, 177001, Himachal Pradesh (H.P.)-India*

ARTICLE HISTORY

Received: January 08, 2020
Revised: March 29, 2020
Accepted: April 09, 2020

DOI:
10.2174/2213476X07666200423081738

Abstract: Ranitidine is a well known H_2 blocker antihistaminic drug used for symptomatic relief of heartburn, indigestion, acid indigestion, peptic ulcer and hyperacidity. However, On 13th September 2019, the United States Food and Drug Administration (USFDA) has given an alerting statement regarding the presence of nitrosamine impurity called N-nitrosodimethylamine (NDMA) in ranitidine containing products. Recently, some pharmaceutical companies have also recalled their ranitidine containing products from the market. Thus, there is a need to understand about these impurities in ranitidine containing products. The first part of this article highlights the mechanism of action of ranitidine in established therapeutic indications along with its adverse drug reactions and contraindications. Further, the introduction of genotoxic impurities in pharmaceutical products along with its types and mechanism of toxicity of ranitidine containing genotoxic impurity have been discussed.

Keywords: Ranitidine; H_2 blocker; genotoxic impurities; N-nitrosodimethylamine.

1. INTRODUCTION

Genotoxic impurities are found in pharmaceutical products. The well known genotoxic impurity in pharmaceutical products is N-nitrosodimethylamine(NDMA) which is classified by the International Agency for Research on Cancer (IARC) as a probable human carcinogen (a substance that could cause cancer). It is found in water and foods, including meats, dairy products, and vegetables at low levels. Recently, On 13th September 2019, Food and Drug Administration (FDA) has given an alerting statement regarding the presence of nitrosamine impurity called N-nitrosodimethylamine (NDMA) in ranitidine containing products.

Ranitidine is an antihistamine drug (H_2 blocker) which was approved by the United States Food and Drug Administration (USFDA) in 1999 for symptomatic relief of heartburn, indigestion, acid, peptic ulcer and hyperacidity by decreasing the amount of acid created in the stomach [1]. It is available in the market in the form of tablets (75mg, 150mg, 300mg), injection (25mg/ml), syrup (15mg/ml), and capsules (150mg, 300mg). The Daily Defined Dose (DDD) of ranitidine is 25 mg/ml (in injection form), 7 mg/5ml (as oral liquid), 150 mg (as tablet). It is used for various indications such as in gastroesophageal reflux disease, imparts relief in heartburn, in gastrointestinal hypersecretory conditions like Zollinger Ellison syndrome, involves eradication of helicobacter pylori which is responsible for duodenal ulcers and in stress-induced ulcer [1]. Over-the-counter (OTC) ranitidine is approved to prevent and relieve heartburn associated with acid ingestion and sour stomach. Ranitidine contains a furan ring in its structure as shown in Fig. (**1**), with the molecular weight 314.404 g/mol.

2. PHARMACOKINETICS

Most forms of ranitidine are absorbed orally in the stomach due to its predominant unionized form. It is metabolized presystemically in the liver

*Address correspondence to this author at the Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research, Raebareli (NIPER-R)-226002, Lucknow (U.P.)-India; Tel: +91-0522 – 2497903;
E-mail: abitmesra@gmail.com

2213-476X/19 $58.00+.00

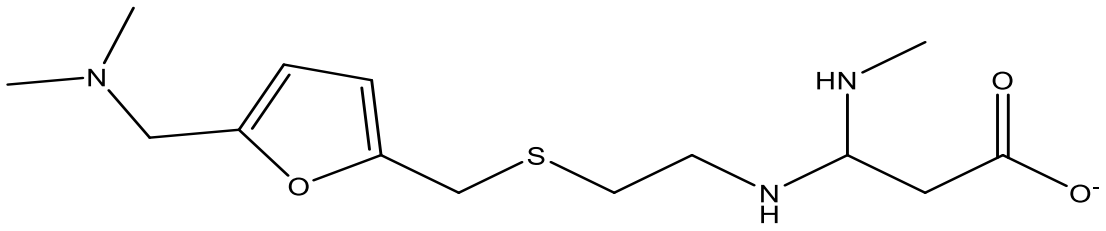

Fig. (1). Structure of ranitidine.

and approximately 50% of it reaches to systemic circulation [2]. In systemic circulation, it binds with albumin and is metabolized in the liver by CYP450 into an active metabolite. Its onset of action is 55-65 minutes. The half-life of oral ranitidine is 2.5-3h. Approximately more than 90% of this drug is excreted in 15-18h through urine [3].

3. MECHANISM OF ACTION OF RANITIDINE IN ESTABLISHED THERAPEUTIC INDICATION

In the gastrointestinal tract, mainly in the stomach, hydrochloric acid is formed by gastric parietal cells. When food enters the stomach, signals are transmitted to the brain that stimulates the vagus nerve which results in the release of acetylcholine and gastrin. The released acetylcholine acts on the muscarinic receptor present in the gastro-intestinal tract (GIT) which stimulates histaminocytes and results in the release of histamine. Further, histamine acts on H_2 receptors present on oxyntic cells and results in the release of H^+ ions. These H^+ ions combine with the chloride ion and there occurs the formation of hydrochloric acid. Acetyl choline also induces release of gastrin that binds to its respective gastrin receptors and results in the release of histamine. Normally, HCl plays a vital role in breakdown, digestion and absorption of nutrients such as proteins. However, excess of HCl damages gastric linings and results in gastric ulcer. Ranitidine is a competitive H_2 antagonist which reduces formed HCL in the stomach as shown in Fig. (**2**) [4].

4. ADVERSE EFFECTS AND CONTRAINDICATIONS

Various adverse drug reactions are associated with the use of ranitidine such as dizziness, insomnia, vertigo, arrhythmia, hepatotoxicity, jaundice, hepatitis, *etc*. [4]. It reduces the absorption of drugs like antifungals, azoles and vitamin B_{12} [3]. It should not be used in combination with the drugs that reduce gastric acid secretion in patient suffering from porphyria as well as in pregnancy. Ranitidine is concerned with bradycardia when administered through i.v route [3].

5. GENOTOXIC IMPURITIES

The synthesis of any drug involves reagents, solvents, catalysts, and other processing aids. As a result of chemical synthesis or subsequent degradation, impurities reside in all drug substances and associated drug products. Impurities are basically substances that have no therapeutic value but have the tendency to cause a harmful effect. Genotoxic impurities are defined as impurities having a tendency to cause mutations in genetic material so that there is a loss of functionality and integrity of cell.

5.1. Types of Genotoxic Impurities

Various genotoxic impurities are nitrosamines, sulfonates, alkyl halides, hydrazines and epoxides which are commonly found in pharmaceutical products. The brief discussion regarding these impurities is given below.

5.1.1. Nitrosamines

These are the chemical compounds having a nitroso group bonded to the amine group. These are reaction byproducts of nitrite and amines at a higher temperature. The well-known nitrosamine containing genotoxic impurity found in pharmaceutical products is N-nitroso dimethylamine (NDMA). The well-known angiotensin receptor blocker i.e. valsartan was recalled from the market in 2018 due to the presence of this impurity [5]. Other nitrosamine impurities found in pharmaceutical products are N-nitrosodiethylamine (NDEA) and N-methyl-4-aminobutyricacid (NMBA) [6].

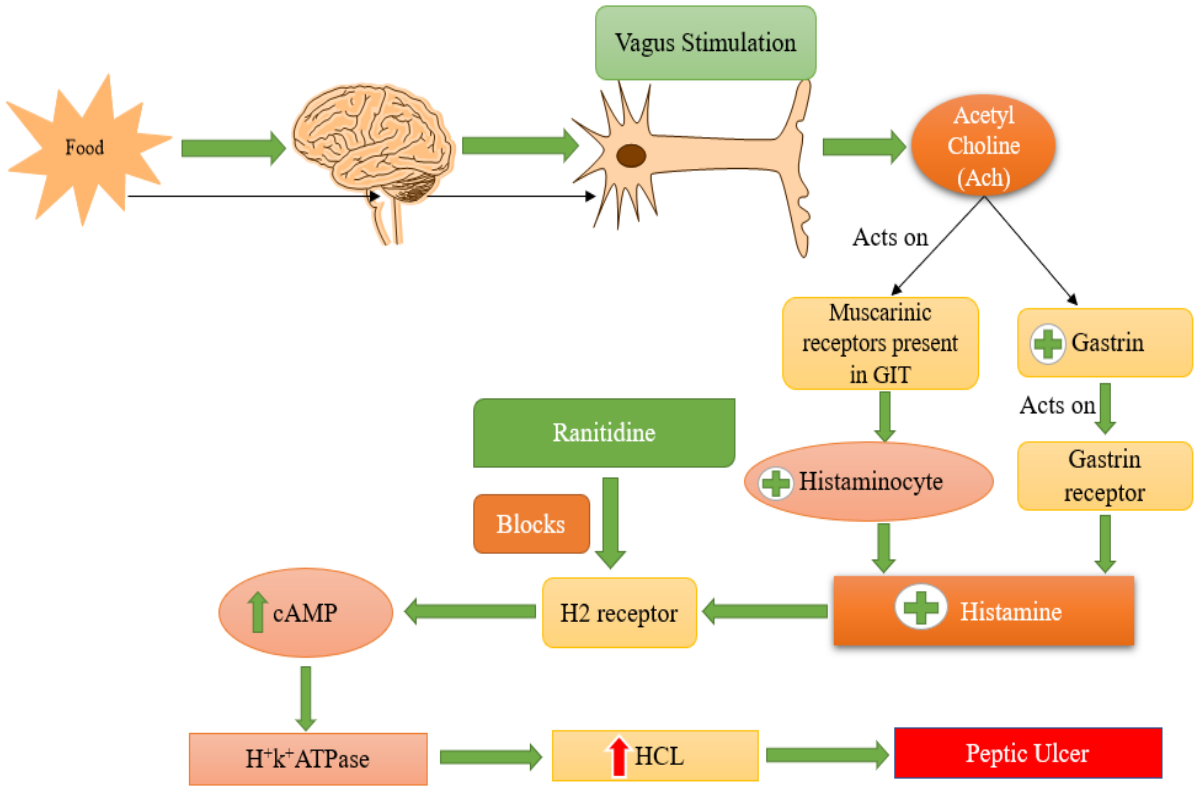

Fig. (2). Mechanism of action of ranitidine in established therapeutic indication. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

5.1.2. Sulfonates

Salt formation is a useful technique for optimizing the physicochemical processing (formulation), biopharmaceutical or therapeutic properties of active pharmaceutical ingredients (APIs), and sulfonate salts are widely used for this purpose. However, sulfonic acids can react with low molecular weight alcohols such as methanol, ethanol, or isopropanol to form the corresponding sulfonate esters which are potential genotoxic and carcinogenic agents.

5.1.3. Alkyl halides

Alkyl halides are also formed during the salt formation in the drug formulation process [7,8]. These compounds react with DNA through nucleophilic substitution and result in a mutagenic effect.

5.1.4. Hydrazines

These compounds are used as a starting material in the synthesis of various types of drugs and found to have genotoxic effects. These compounds react with DNA and result in the formation of methyldiazonium and methyl free radicals, inducing gene mutations [8,9].

5.1.5. Epoxides

Epoxides are another genotoxic impurities found in pharmaceutical products. These are the electrophilic alkylating compounds which have the tendency to react with DNA directly [10-12].

Various tests are available to check the genotoxicity of chemicals such as bacterial Ames test, comet assay, and cytogenetic assays such as micronucleus and chromosomal aberration assays, *etc*.

6. GENOTOXIC IMPURITY IN RANITIDINE CONTAINING PRODUCTS

Recently, nitrosamine impurity *i.e.* NDMA in ranitidine has been highlighted by the USFDA as an area of concern. This impurity is classified as a probable human carcinogen and is believed to have been introduced into the finished products as a result of the manufacturing process.

Table 1. Recall of ranitidine containing products from the market [13].

Sr. no.	Company Name	Brand Name(s)	Recall Date	Product Description
1.	American Health Packaging	American Health Packaging	27th Feb 2020	Ranitidine Tablets, USP 150mg
2.	Denton Pharma, Inc. dba Northwind Pharmaceuticals	Northwind	8th Jan 2020	Ranitidine Tablets 150mg and 300mg
3.	Appco Pharma LLC	Ani	7th Jan 2020	Ranitidine Tablets 150mg and 300mg
4.	Glenmark Pharmaceuticals, Inc.	Glenmark	17th Dec 2019	Ranitidine Tablets 150mg and 300mg
5.	Amneal Pharmaceuticals, LLC	Amneal	22nd Nov 2019	Ranitidine Tablets, 150 mg and 300 mg, and Ranitidine Syrup (Ranitidine Oral Solution, USP), 15 mg/mL
6.	Precision Dose Inc.	PrecisionDose	19th Nov 2019	Ranitidine Oral Solution, USP 150 mg/10 mL
7.	GSMS, Inc.	GSMS Incorporated	15th Nov 2019	Ranitidine HCl 150mg and 300mg Capsules
8.	American Health Packaging	AHP	8th Nov 2019	Ranitidine Liquid Unit Dose Cups
9.	Aurobindo Pharma USA, Inc.	Aurobindo & DG Health	6th Nov 2019	Ranitidine
10.	Novitium Pharma	Novitium Pharma	25th October 2019	Ranitidine Hydrochloride Capsules 150 mg and 300 mg
11.	Lannett Company, Inc.	Lannett Company, Inc.	25th October 2019	Ranitidine Syrup (Ranitidine Oral Solution, USP), 15mg/mL
12.	Dr. Reddy's Laboratories, Ltd.	Dr. Reddy's, Kroger, Walgreens, and others	23rd October 2019	Ranitidine Tablets & Capsules
13.	Perrigo Company plc	Perrigo Company plc	23rd October 2019	Ranitidine (all pack sizes)
14.	Apotex Corp.	Apotex Corp.	25th September 2019	Ranitidine Tablets 75mg and 150mg
15.	Sandoz Inc.	Sandoz Inc.	23rd September 2019	Ranitidine Hydrochloride Capsules

6.1. Mechanism of Toxicity of NDMA

N-nitroso dimethylamine (NDMA) is metabolized in the liver by enzymatic cytochrome P-4502E1 into hydroxy methyl nitrosamine. This hydroxy methyl nitrosamine under non-enzymatic conditions is converted into methyl diazonium ion and formaldehyde. Further, methyl diazonium ion undergoes methylation of macromolecules such as proteins and hepatocytic DNA[14-16]. The formed formaldehyde is reduced into methanol which results in liver toxicity and activation of the inflammatory signaling pathway as shown in Fig. (**3**). These processes generate extreme oxidative stress which causes hepatocyte damage and necrosis[17-19].

N-nitroso dimethylamine (NDMA) also favors the entry of bacterial endotoxin such as lipopolysaccharide (LPS) due to increased gut permeability. Basically, LPS is a component cell wall of gram-negative bacteria which activates Kupffer cells [20,21]. These activated Kupffer cells produce cytokines and tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1) that further activates hepatic stellate cells [22-24]. NDMA also activates lymphocytes that produce several inflammatory cytokines such as IL1β, IL13, IL6, TNF-α and TGFβ1, and further there is direct activation of stellate cells [25-27]. In addition, there is sinusoidal endothelial cell damage due to which there is a production of factors such as connective tissues growth factor-1 (CTGF) and fibroblast growth factor-10 (FGF-1) [28-30]. Due to the damage of sinusoidal endothelial cells, there is a release of Factor VII which is responsible for the platelet aggregation which further produces

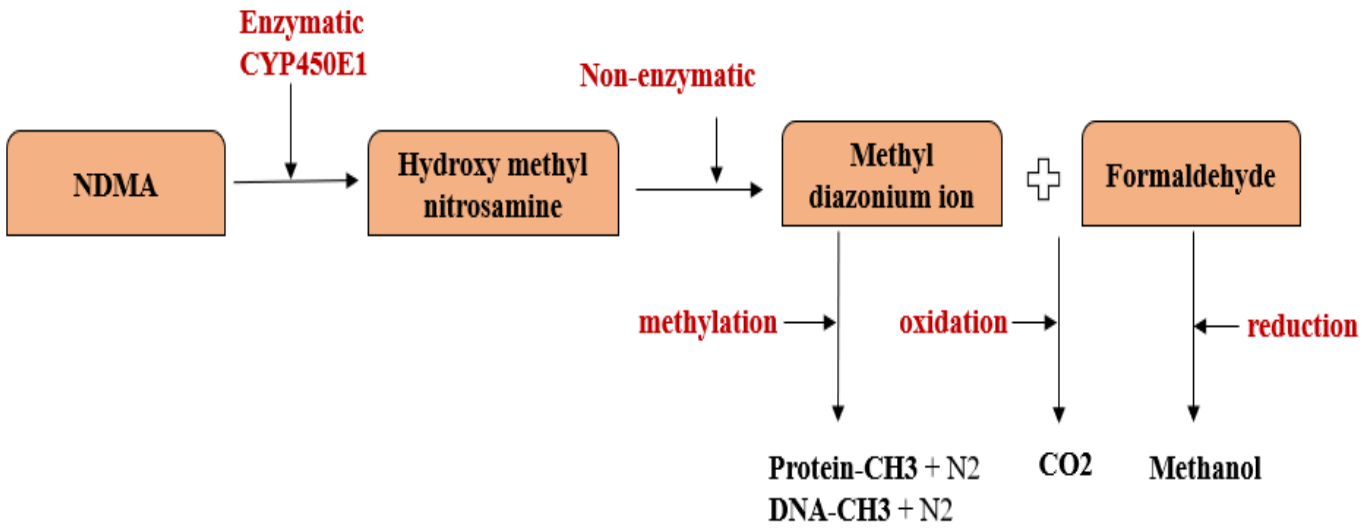

Fig. (3). Metabolic degradation of N-nitroso dimethylamine (NDMA) in the liver. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

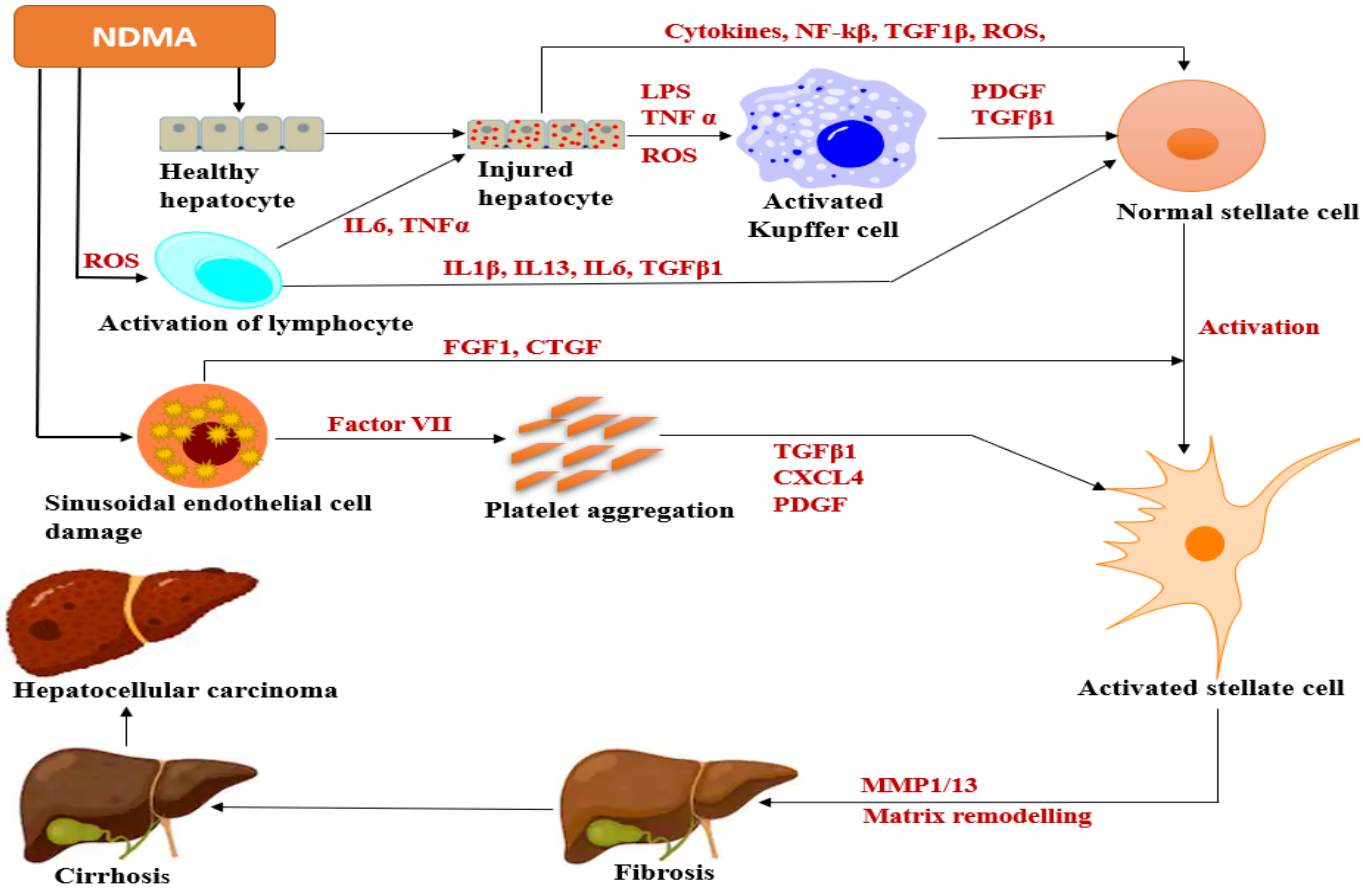

Fig. (4). Mechanisms involved in NDMA induced hepatic fibrosis. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

TGF-β1, platelet-derived growth factor (PDGF) and CXC chemokine ligand 4 (CXCL4) [31,32]. There are major interstitial collagenases which are present in human as Matrix metalloproteinase-1 (MMP-1), MMP-13. These MMPs have a tendency to degrade matrix proteins as collagens and it plays an important role in remodeling of connective tissue matrix during the pathogenesis of hepatic fibrosis [17]. Hepatic fibrosis results in cirrhosis, carcinoma and death as shown in Fig. (**4**).

CONCLUSION

In conclusion, genotoxic impurities are formed during the synthesis of drugs which could result in mutations or can cause cancer. These impurities should be in acceptable limits in various pharmaceutical products and should be monitored by the regulatory bodies. The NDMA level in ranitidine containing products was checked by the U.S. FDA by conducting various tests in the stomach and intestine environment and no NDMA formation was found during these processes. In addition, the U.S. FDA has advised companies to voluntarily recall the ranitidine batches if it crosses the permissible limits (96 ng or 0.32 parts per million per day) of NDMA but on the other side, if the levels of NDMA lie in the acceptable limit then there is no need to recall the drug and it will be considered as safe [33].

CONFLICTS OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content of this manuscript.

FUNDING

All the authors acknowledge the financial assistance received from the Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers, Government of India for carrying out this work. The NIPER communication number of this manuscript is NIPER-R/ Communication/120.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. S.J.S. Flora, Director, NIPER, Raebareli for their constant support and motivation.

REFERENCES

[1] Brogden RN, Carmine AA, Heel RC, Speight TM, Avery GS. Ranitidine: a review of its pharmacology and therapeutic use in peptic ulcer disease and other allied diseases. Drugs 1982; 24(4): 267-303. http://dx.doi.org/10.2165/00003495-198224040-00002 PMID: 6128216

[2] Zeldis JB, Friedman LS, Isselbacher KJ. Ranitidine: a new H2-receptor antagonist. N Engl J Med 1983; 309(22): 1368-73. http://dx.doi.org/10.1056/NEJM198312013092206 PMID: 6314139

[3] Carey PF, Martin LE, Owen PE. Determination of ranitidine and its metabolites in human urine by reversed-phase ion-pair high-performance liquid chromatography. J Chromatogr A 1981; 225(1): 161-8. http://dx.doi.org/10.1016/S0378-4347(00)80255-8 PMID: 6271798

[4] Weingart J, Kunert H, Ottenjann R. Stimulation of gastric acid secretion by intravenous amino acid infusion and its inhibition by H2-receptor antagonists ranitidine and cimetidine. Br J Clin Pharmacol 1980; 10(2): 174-5. http://dx.doi.org/10.1111/j.1365-2125.1980.tb01739.x PMID: 6107119

[5] Pottegård A, Kristensen KB, Ernst MT, Johansen NB, Quartarolo P, Hallas J. Use of N-nitrosodimethylamine (NDMA) contaminated valsartan products and risk of cancer: Danish nationwide cohort study. bmj 2018; 362: k3851.

[6] Verna L, Whysner J, Williams GM. N-nitrosodiethylamine mechanistic data and risk assessment: bioactivation, DNA-adduct formation, mutagenicity, and tumor initiation. Pharmacol Ther 1996; 71(1-2): 57-81. http://dx.doi.org/10.1016/0163-7258(96)00062-9 PMID: 8910949

[7] Sobol Z, Engel ME, Rubitski E, Ku WW, Aubrecht J, Schiestl RH. Genotoxicity profiles of common alkyl halides and esters with alkylating activity. Mutat Res 2007; 633(2): 80-94. http://dx.doi.org/10.1016/j.mrgentox.2007.05.004 PMID: 17644026

[8] Elder DP, Lipczynski AM, Teasdale A. Control and analysis of alkyl and benzyl halides and other related reactive organohalides as potential genotoxic impurities in active pharmaceutical ingredients (APIs). J Pharm Biomed Anal 2008; 48(3): 497-507. http://dx.doi.org/10.1016/j.jpba.2008.06.009 PMID: 18657926

[9] Bercu JP, Dobo KL, Gocke E, McGovern TJ. Overview of genotoxic impurities in pharmaceutical development. Int J Toxicol 2009; 28(6): 468-78. http://dx.doi.org/10.1177/1091581809349195 PMID: 19966139

[10] Snodin DJ. Genotoxic impurities: from structural alerts to qualification. Org Process Res Dev 2010; 14(4): 960-76. http://dx.doi.org/10.1021/op100118e

[11] De Flora S, Zanacchi P, Camoirano A, Bennicelli C, Badolati GS. Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutat Res 1984; 133(3): 161-98. http://dx.doi.org/10.1016/0165-1110(84)90016-2 PMID: 6374443

[12] Elder DP, Snodin D, Teasdale A. Analytical approaches for the detection of epoxides and hydroperoxides in active pharmaceutical ingredients, drug products and herbals. J Pharm Biomed Anal 2010; 51(5): 1015-23. http://dx.doi.org/10.1016/j.jpba.2009.11.023 PMID: 20031361

[13] https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts

[14] Fan G, Tu Y, Chen C, Sun H, Wan C, Cai X. DNA methylation biomarkers for hepatocellular carcinoma. Cancer Cell Int 2018; 18(1): 140. http://dx.doi.org/10.1186/s12935-018-0629-5 PMID: 30245591

[15] Zheng YF, Lu X, Zhang XY, Guan BG. The landscape of DNA methylation in hepatocellular carcinoma. J Cell Physiol 2019; 234(3): 2631-8.

http://dx.doi.org/10.1002/jcp.27077 PMID: 30145793

[16] Villanueva A, Portela A, Sayols S, *et al*. HEPTROMIC Consortium. DNA methylation-based prognosis and epidrivers in hepatocellular carcinoma. Hepatology 2015; 61(6): 1945-56. http://dx.doi.org/10.1002/hep.27732 PMID: 25645722

[17] George J, Tsuchishima M, Tsutsumi M. Molecular mechanisms in the pathogenesis of N-nitrosodimethylamine induced hepatic fibrosis. Cell Death Dis 2019; 10(1): 18. http://dx.doi.org/10.1038/s41419-018-1272-8 PMID: 30622238

[18] Li P, He K, Li J, Liu Z, Gong J. The role of Kupffer cells in hepatic diseases. Mol Immunol 2017; 85: 222-9. http://dx.doi.org/10.1016/j.molimm.2017.02.018 PMID: 28314211

[19] Luedde T, Schwabe RF. NF-κB in the liver--linking injury, fibrosis and hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2011; 8(2): 108-18. http://dx.doi.org/10.1038/nrgastro.2010.213 PMID: 21293511

[20] Liu H, Liao R, He K, Zhu X, Li P, Gong J. The SMAC mimetic birinapant attenuates lipopolysaccharide-induced liver injury by inhibiting the tumor necrosis factor receptor-associated factor 3 degradation in Kupffer cells. Immunol Lett 2017; 185: 79-83. http://dx.doi.org/10.1016/j.imlet.2017.02.016 PMID: 28286228

[21] Wheeler MD. Endotoxin and Kupffer cell activation in alcoholic liver disease. Alcohol Res Health 2003; 27(4): 300-6.
PMID: 15540801

[22] Mekala S, Tulimilli SV, Geesala R, Manupati K, Dhoke NR, Das A. Cellular crosstalk mediated by platelet-derived growth factor BB and transforming growth factor β during hepatic injury activates hepatic stellate cells. Can J Physiol Pharmacol 2018; 96(8): 728-41. http://dx.doi.org/10.1139/cjpp-2017-0768 PMID: 29558627

[23] Kiagiadaki F, Kampa M, Voumvouraki A, Castanas E, Kouroumalis E, Notas G. Activin-A causes Hepatic stellate cell activation via the induction of TNFα and TGFβ in Kupffer cells. Biochim Biophys Acta Mol Basis Dis 2018; 1864(3): 891-9. http://dx.doi.org/10.1016/j.bbadis.2017.12.031 PMID: 29287776

[24] Gressner AM. The cell biology of liver fibrogenesis - an imbalance of proliferation, growth arrest and apoptosis of myofibroblasts. Cell Tissue Res 1998; 292(3): 447-52. http://dx.doi.org/10.1007/s004410051073 PMID: 9582401

[25] Wang L, Tu L, Zhang J, Xu K, Qian W. Stellate cell activation and imbalanced expression of TGF-β1/TGF-β3 in acute autoimmune liver lesions induced by ConA in mice. Bio-Med Res Int 2017; 1-12. http://dx.doi.org/10.1155/2017/2540540

[26] Meng F, Wang K, Aoyama T, *et al*. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. Gastroenterology 2012; 143(3): 765-776.e3. http://dx.doi.org/10.1053/j.gastro.2012.05.049 PMID: 22687286

[27] Holt AP, Salmon M, Buckley CD, Adams DH. Immune interactions in hepatic fibrosis. Clin Liver Dis 2008; 12(4): 861-882, x. http://dx.doi.org/10.1016/j.cld.2008.07.002 PMID: 18984471

[28] Ruart M, Chavarria L, Campreciós G, *et al*. Impaired endothelial autophagy promotes liver fibrosis by aggravating the oxidative stress response during acute liver injury. J Hepatol 2019; 70(3): 458-69. http://dx.doi.org/10.1016/j.jhep.2018.10.015 PMID: 30367898

[29] DeLeve LD. Liver sinusoidal endothelial cells and liver regeneration. The Journal ofclinical investigation 2013; 123(5): 1861-6. http://dx.doi.org/10.1172/JCI66025

[30] Xie G, Choi SS, Syn WK, *et al*. Hedgehog signalling regulates liver sinusoidal endothelial cell capillarisation. Gut 2013; 62(2): 299-309. http://dx.doi.org/10.1136/gutjnl-2011-301494 PMID: 22362915

[31] Burt AD. C. L. Oakley Lecture (1993). Cellular and molecular aspects of hepatic fibrosis. J Pathol 1993; 170(2): 105-14. http://dx.doi.org/10.1002/path.1711700203 PMID: 8345406

[32] Zaldivar MM, Pauels K, von Hundelshausen P, *et al*. CXC chemokine ligand 4 (Cxcl4) is a platelet-derived mediator of experimental liver fibrosis. Hepatology 2010; 51(4): 1345-53. http://dx.doi.org/10.1002/hep.23435 PMID: 20162727

[33] FDA Updates and Press Announcements on NDMA in Zantac (ranitidine). Available at: https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-zantac-ranitidine