ORIGINAL ARTICLE

Inflection of Oxidative Status of Nepthalene Induced Liver Injury in Mice

IRAM ARSHAD[1], ANAM ARSHAD[2], MUHAMMAD HUMAYUN[3], *ARIF MALIK[4]*
[1,2]Senior Demonstrator Biochemistry, Central Park Medical College, Lahore
[3]HOD Biochemistry Department, Central Park Medical College, Lahore, Mobile: 03009439748
[4]Institute of Molecular Biology and Biotechnology, The University of Lahore-Pakistan
Correspondence to Dr. Iram Arshad, Email: iram.arshad04@gmail.com, Cell: 03244255998

ABSTRACT

Background: Cancer is a leading cause of morbidity and mortality worldwide. Numbers of therapeutic strategies are being used to target aberrant pathways involved in malignant cell proliferation and survival. The increasing side effects and evolution of resistant population has driven scientists to device newer and targeted therapeutic options to be used.
Aim: To investigate the hepatotoxic effect of intraperitoneal administration of naphthalene derivatives in mice.
Methods: Naphthalene derivative (Naphthoquinone) was dissolved in DMSO (Dimethyl Sulfoxide) and given to the white albino mice to compare it with control group after 30 days of administration. The fifteen mice used for the experiment were divided into 3 groups (A, B, C). Group B and C received 50 mg/kg and 150 mg/kg per body weight intraperitoneally respectively, whereas control group (A) received no drug. Biochemical assessment of ALT, AST, ALP, Albumin, Cholesterol and triglycerides were done by kit method, histological analysis was done and stress markers (MDA, GSH, SOD, and Catalase) were analyzed using spectrophotometer.
Results: Naphthalene derivative toxicity was observed as the result showed that mean serum ALT, AST and ALP level were higher in mice administered naphthalene derivative as compared to control group. In addition, histological changes such as inflammation leading to cirrhosis were seen in liver on different doses, other biomarkers of toxic effects include glutathione depletion, lipid peroxidation, DNA fragmentation and the production of reactive oxygen species (ROS).
Conclusion: We concluded that naphthalene derivatives metabolism triggers production of ROS, coupled with impaired oxidant/antioxidant balance, leading to a state of oxidative-stress that could have been partially responsible for the slight hepatotoxicity and the disturbance in the level of hepatic enzymes seen in this study. Therefore, a possible conclusion that such biochemical changes observed in these experimental animals may be seen in human beings is undeniable so naphthalene derivatives may have more toxic effects than therapeutic effects.
Keywords: Hepatotoxicity, Oxidative Stress, Antioxidants, Naphthalene, Naphthoquinone, DMSO

INTRODUCTION

Cancer is the irresistible growth of abnormal cell. There are different types of cancer that affect different organs (Revathi and Prashanth 2015). Cancer is an advanced disease includes various physiological changes in the cell that eventually causes malignancy(Seyfried and Shelton 2010). Carcinogenesis is a multistep process caused by alterations in the gene that activate signal transduction pathways and transform the normal cell into the cancerous cell(Fantini et al. 2015). The past decades have showed that cancer is treatable with the help of chemotherapy, radiotherapy and surgery. Combination therapy and early diagnosis can improve survival(Shewach and Kuchta 2009). Chemotherapy includes various subtypes according to their mode of action: antibiotics, topoisomerase I inhibitors, topoisomerase II inhibitor, antimetabolites, alkylating agents, mitosis inhibitors. Adverse effects of chemotherapy can be classified into long term and short term effects(Goetz et al. 2003).

Naphthalene is a two ringed aromatic hydrocarbon and its derivatives (naphthoquinone) exert antimicrobial, insecticidal and wound healing effects and serve as potential anticancer agent(Qiu et al. 2018). Naphthoquinones are highly attracted for anticancer therapies. However due to their potential side effects and toxicity, their use in anticancer therapies is limited(Di Yang et al. 2019). Naphthoquinone can cause lipid peroxidation, glutathione depletion and excessive production of the reactive oxygen species(Bagchi et al. 2002a). Reactive oxygen species (ROS) in normal concentration plays important role in maintaining various physiological mechanisms, which includes, apoptosis, cell proliferation, cell differentiation and modulation of signal pathways.

Received on 27-11-2019
Accepted on 13-04-2020

Excessive production of ROS induce damage such as gene mutations, cell death, chromosomal breakage and carcinogenesis(Cerutti 1985). Mammalian cells contain three basic antioxidant enzymes that are considered to be essential for life in all metabolizing cells. These enzymes include superoxide dismutase (SOD), catalase and glutathione peroxidase(Weydert and Cullen 2010).

The aim of this study was to evaluate the toxic effect of naphthalene derivatives used as anticancer drugs to normal cells of mice.

MATERIALS AND METHODS

The study was conducted at Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore. All animal procedures and experimental protocols were approved by The University of Lahore Animal Care and Use Council.
Drug preparation: Naphthoquinone was dissolved in DMSO and a stock solution of 50mg/ml was made. Doses were set to suitable volumes, maximum of 0.5ml intraperitoneally in mice.
Animal and environmental conditions: White albino mice (weighing between 20-35 grams) were acquired from Animal House of the University of Lahore. All mice were stored in large airy cages in groups of 5 mice per cage under controlled condition of temperature 25C and normal photoperiod (12 hours' dark and light) , and free access to water and food throughout the experiment.
Pilot study: Pilot study was done using small number of animals (two each group). Four doses were choose and given to four groups of albino mice to determine the intraperitoneal LD50. All four groups of mice were given 40 mg/kg, 67 mg/kg, 100 mg/kg and 200 mg/kg of drug mixture respectively. The location for the intraperitoneal injection was lower left abdominal quadrant. The animals were observed for first 2 hours and then at 6th and 24th hour for any toxic symptoms. After 24 hours' number of deceased mice were counted in

each group. In animals receiving intraperitoneal injection, abdominal muscle contraction, ataxia, altered breathing and heartrate pattern and itching was observed, which persisted for few hours. At the 6th hour they were drowsy and less responsive. The severity of these effects was related to the level of dose. However, at 24th hour most of the survivors had recovered from these symptoms, but alopecia was observed in the animals with high dose. LD50 of drug mixture was found to be 200mg/kg by the number of mice died at each dose.

Study design: Experimental type of study was conducted using animal model. 15 adult (8 week old) male and female albino mice were purchased and randomly allocated into three groups, five mice each (n=5). Before assigning to groups, the weight difference of the animals used must not exceed 20% of mean weight. Group A (G-A), control group includes mice with no drug; Group B (G-B) includes mice that were injected i.p 50 mg/kg of drug mixture; Group C (G-C) includes mice that were injected i.p 150 mg/kg of drug mixture. The maximum tolerated dose (MTD) in this study is defined as the highest dose that will be tolerated and not produce major life threatening toxicity for the study duration. After intraperitoneal injection of drug mixture on selected doses, mice were observed for 30 days. After 30 days all the animals were weighed before and after the treatment. The decrease in the body weight before and after the treatment was neglect able. The animals were weighed, anesthetized with chloroform, necropsied, and discarded after collection of all organs and blood samples to analyze the hematological, biochemical and histological parameters. Blood samples directly from heart of all mice were collected in syringes for evaluation of liver function test (LFTs), lipid profile. Liver were removed and weighed from each mouse and divided into two halves, one half was preserved in 10%buffered formalin for histological analysis by light microscopy and other half was stored at -80 C in separate falcon tubes for oxidative stress tests.

Table 1: Group designation and dose level

Groups	Doses
A	Control
B	Naphthoquinone @ 50 mg/kg/bw t i.p
C	Naphthoquinone @ 150 mg/kg/bw t i.p

Method: Mice were divided into three groups and each group comprises five animals. Group A was treated as control. Group B and C were treated with Naphthoquinone 50 mg/kg/bwt i.p and 150 mg/kg/bwt i.p respectively for 30 days. The serum was obtained after centrifugation at 5000rpm for 5 min each at 4C. Serum analysis was done to determine the serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) to assess hepatobiliary function by kit method. Serum cholesterol and triglycerides were assessed to determine the lipid profile. Moreover, histopathological examination of liver was also performed. Tissue were preserved in 10% formalin and imbedded in paraffin blocks, and were cut into 5micrometer thick slices. Hematoxylin-eosin staining was done. The bright-field images were obtained using microscope(Tamaki et al. 2003). 60-500mg of sample was taken from each tissue separately depending on the organ size. The samples were then homogenized in 50 mg/ml of 0.5 M phosphate buffer solution with pH 7.2 and then centrifuged at 5000 rpm for complete 10 minutes each. The clear supernatant was separated and used for the testing. Superoxide dismutase (SOD) activity was determined by the method of kakkar(Kakkar et al. 1995). MDA was estimated by Beuge and Aust method(Buege and Aust 1978). Catalase activity was measured by the method of Aebi(Aebi 1984). Glutathione (GSH) activity was conducted by the Spectrophotometric/microplate reader assay method.

Statistical analysis: The statistical analysis was conducted on the data using statistical software package (SPSS version 22) for windows. The obtained data was entered on SPSS version 22. Furthermore, the tables and graphs showing the results were generated on SPSS and Microsoft Excel 2013. One-way analysis of variance (ANOVA) and independent samples t-test was used to compare the data. The probability $p< 0.05$ was considered significant. The correlations were calculated between the groups.

RESULTS

Table 1 shows that Naphthoquinone have highly significantly effect on ALT, AST, and ALP levels in serum. The administration of naphthoquinone 50 mg/kg and 150 mg/kg/bwt in mice leads to significant increase of biochemical marker. AST (41.5±11.24 U/L, 68.75±20.38 U/L), ALT (35±6.0 U/L, 80.5±31.84 U/L) and ALP (224.75±111.52 U/L, 211.25±68.94 U/L) was reported in both groups G-B and G-C respectively as compared to control (36±15.232 U/L), (24.5±9.98 U/L), (469.5±145.04 U/L) respectively and representing acute hepatocytes damage. In case of albumin in naphthoquinone treated group G-B (3.925±0.4717 g/L) and G-C (4.45±0.68 g/L) was statistically significant increased ($P<0.05$) as compared to control (3.65±0.52 g/L). According to the results of table 2 the administration of naphthoquinone 50 mg/kg/bwt in mice induced increase MDA levels was reported in group B (9.16±9.14b) and C (8.61±3.79a) as compared to control group A (0.93±1.52c). The lowest value of SOD (62.28±1b), GSH (5.93±0.58b) and CAT (24.59±3.51b) in mice was reported in group C receiving naphthoquinone at the doses of 150 mg/kg/bwt and has a significant difference with control group (79.87±1.62a), (8.68±0.54a), (32.58±3.19a) respectively. MDA has inverse correlation (Table 3) with SOD and GSH $r= -.925^{**}$ ($P= .000$) and $r= -.782^{**}$ ($P= .003$) respectively. MDA has positive correlation with ALT $r= .791^{**}$ ($P= .002$). Similarly SOD has positive correlation with GSH r=.886 ($P= .000$). No clear relationship between SOD and CAT $r= .532$ ($P= .075$) and naphthalene tolerance of albino mice examined here, it would possible to be drawn. The histology of liver section from the control shows normal architectural appearance. The cords of hepatocytes are arranged symmetrically from the central vein to the periphery of the hepatic lobules. Microscopy imaging revealed the normal appearance of parenchyma and liver extracellular matrix. Hepatocytes are arranged in cords located between sinusoidal capillaries. Polygonal and mononucleotide hepatocytes. No signs of apoptosis and necrosis is present. Naphthoquinone low dose treated group shown loss of cellular boundaries, mildly congested central vein, hypertrophic hepatocytes and binucleated large nuclei with obvious increase in inflammatory cell infiltration around portal vein. Mild nodular formation is also seen but that is close to normal. Naphthoquinone high doses treated group shows cirrhotic changes in liver parenchyma and undistinguished hepatocytes with marked nodular formation.

Table 2: Biochemical variables of liver profile in mice under naphthalene induced hepatic insult

Groups	ALT U/L	AST U/L	ALP U/L	Albumin g/L
G-A	24.5±9.983	36±7.23	469.5±145.046	2.15±0.52
G-B	35±6.0	41.5±11.24	224.75±111.527	3.925±0.47
G-C	80.5±31.84	68.75±20.38	211.25±68.94	4.45±0.68
p-value	0.016	0.031	0.020	0.046

Table 3: Biochemical variables of oxidative stress in mice under naphthalene induced hepatic insult

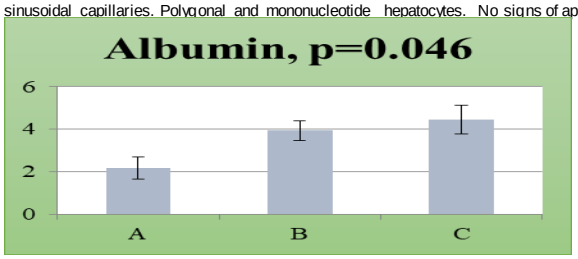
Groups	Catalase	MDA	SOD	GSH
A	32.58±3.19a	1.52±0.93c	79.87±1.62a	8.68±0.54a
B	17.03±3.36c	9.16±0.914b	42.26±2.64c	4.28±1c
C	24.59±3.51b	8.61±1.79a	62.28±1b	5.93±0.58b
P values	0.032***	0.011***	0.010***	0.023***

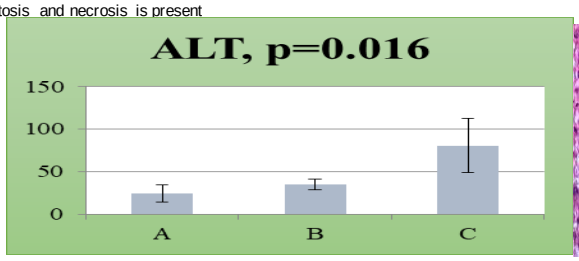
aaTable 4: Pearson S' correlation matrix of different variables under naphthalene induced oxidative stress in mice

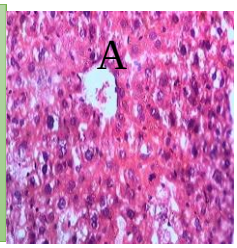
	ALT	AST	ALP	TP	GSH	SOD	MDA	Catalase
ALT	1.000	.418	.832	-.561	-.851	-.863	.791	-.529
		.176	.001	.058	.000	.000	.002	.077
AST		1.000	.511	-.484	-.396	-.385	.544	-.425
			.089	.111	.202	.216	.068	.169
ALP			1.000	-.580*	-.893**	-.944**	.960**	-.473
				.048	.000	.000	.000	.121
TP				1.000	.386	.636*	-.629*	.222
					.216	.026	.029	.488
GSH					1.000	.886**	-.782**	.653*
						.000	.003	.021
SOD						1.000	-.925**	.532
							.000	.075
MDA							1.000	-.358
								.253
Catalase								1.000

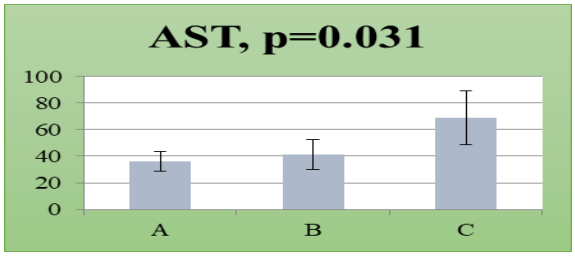
** Correlation is significant at the 0.01 level (2-tailed) * Correlation is significant at the 0.05 level (2-tailed)

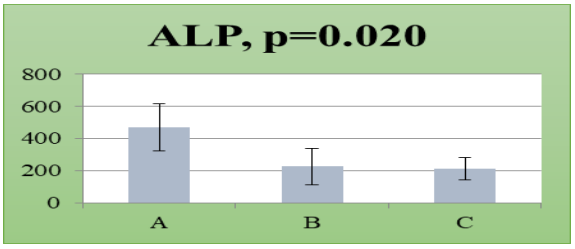
Figure1: (A) The histology of liver section from the control shows normal architectural appearance. The cords of hepatocytes are arranged symmetrically from the central vein to the periphery of the hepatic lobules. Microscopy imaging revealed the normal appearance of parenchyma and liver extracellular matrix. Hepatocytes are arranged in cords located between sinusoidal capillaries. Polygonal and mononucleotide hepatocytes. No signs of apoptosis and necrosis is present

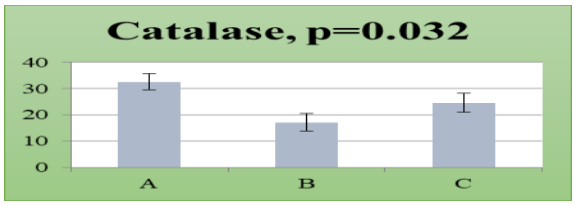


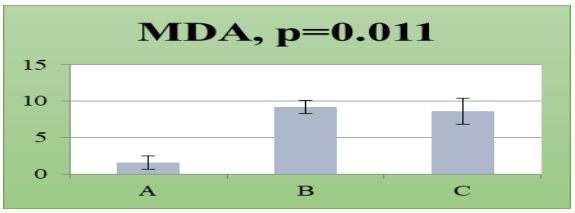


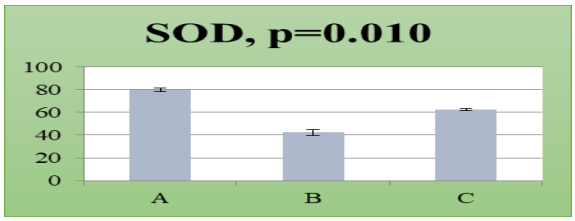


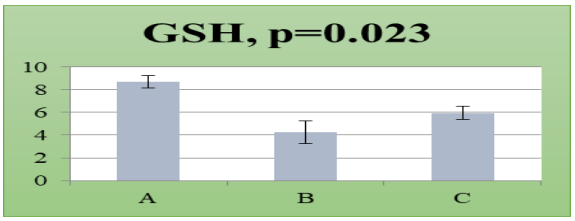












. Figure 2: (B) Naphthalene low dose treated group shown loss of cellular boundaries and mildly congested central vein. (C) Hypertrophic hepatocytes, binucleated large nuclei with prominent nucleoli. (D) Obvious increase in inflammatory cell infiltration around portal vein area.

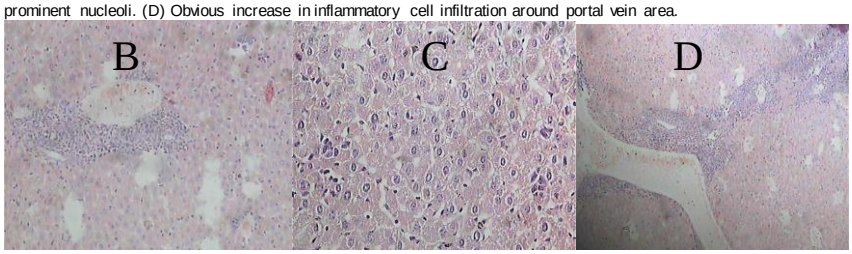


Figure 3: (E) Naphthalene high doses treated group shows cirrhotic changes in liver parenchyma and undistinguished hepatocyte structures seen. (F) Marked nodular formation with cellular necrosis. (G) Nodular formation.

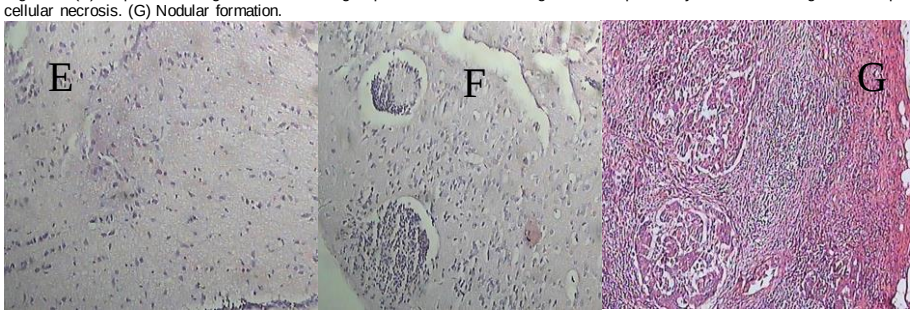
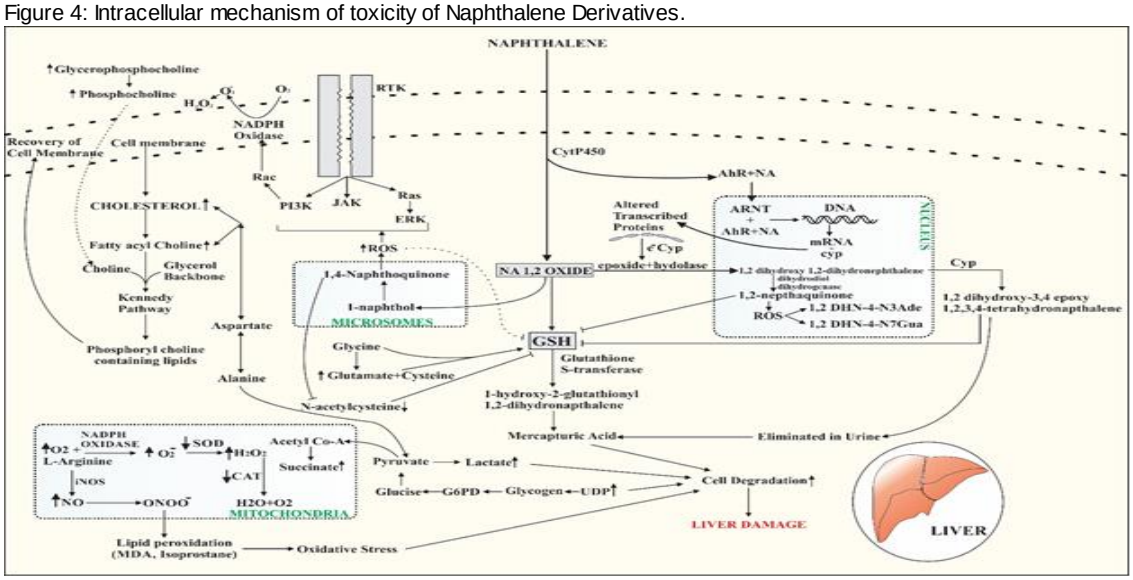


Figure 4: Intracellular mechanism of toxicity of Naphthalene Derivatives.



DISCUSSION

Cancer is a complex disease including various mutations in the cell physiology, eventually leading to malignancies. The biological endpoint of the disease is abnormal cell growth (neoplasia). The anticancer drugs act at various levels such as endothelium, extracellular matrix, cancer cells and the host cells. The tumor cells are targeted by targeting its DNA, RNA or at protein levels. The target of interest for many chemotherapeutic agents are tumor DNA(Espinosa et al. 2003).

Chemotherapeutic agents such as Cisplatin, 5-FU and doxorubicin have been widely used for liver and other cancers(Yuan et al. 2008). However, along with their therapeutic effects in chemotherapy, they also exhibit toxicities and side effects(Ajani 2008). The liver have special role in metabolism. The liver perform many functions in the body that includes detoxification, red blood cell decomposition, glycogen storage, and protein synthesis and production of hormones. Naphthalene is a cyclic hydrocarbon. Naphthalene has shown to react readily with nitrogen oxide and hydroxyl radicals(Preuss et al. 2003). Phthalic anhydride, insect repellent and pharmaceuticals uses naphthalene as their intermediate compound(West et al. 2001). In vivo studies have shown that naphthalene causes cellular destruction and ultrastructural changes like cytoplasmic blabbing, swelling of smooth endoplasmic reticulum and depletion of intracellular glutathione(Plopper et al. 2001). As cytochrome P450 is bio transformed to naphthalene, it produces imbalance in homeostasis and hepatic injury. Following naphthalene treatment the glycerophosphocholine and plasma choline increases, Phosphoryl choline (PCs) is a class of lipids that are mandatory in cell life as a component of membrane bilayer or secondary messenger. Lipids are important component of membrane, metabolic regulators, inter and intra cellular signaling and energy supplements. Naphthalene induced toxicity can be monitored by monitoring change in the lipids(Mencarelli and Martinez–Martinez 2013).

In our study, we used intraperitoneal injection, the liver played critical role in detoxifying reactive intermediate

metabolites as drugs reached liver in relatively short time by this route. Naphthalene is a probable source of free radicals. Rate limiting step in lipid peroxidation is free radical formation(Barouki and Morel 2001). Our study results shows elevation in mean serum ALT, AST, ALP activities and albumin levels at different naphthalene derivatives concentration are indicative of hepatic dysfunction and cholestasis, which may be pronounced at prolonged administration. Radical-mediated lipid peroxidation of liver cell membrane is reflected by elevation in serum AST and ALT(Ray et al. 1999). In the present study, naphthalene-induced hepatotoxicity is followed by an increased hepatic lipid peroxidation (MDA) and decreased GSH contents. This suggests a clear relation between oxidative stress and lipid peroxidation following naphthalene-induced hepatotoxicity. Lipid peroxidation is an important marker of oxidative damage to the biological tissues(Bagchi et al. 2002b).

Naphthoquinone low dose treated group shown obvious increase in inflammatory cell infiltration around portal vein area, loss of cellular boundaries, hypertrophic hepatocytes, binucleated large nuclei with prominent nucleoli and mildly congested central vein. Naphthoquinone high dose treated group animal shows marked nodular formation and cellular necrosis. Majorly shows cirrhotic changes in liver parenchyma and undistinguished hepatocyte structures. In addition, massive fatty degeneration, cellular necrosis, moderate increase of inflammatory cell, and loss of cellular boundaries were observed. Naphthoquinone has a deleterious effect on serum anti oxidative status.

CONCLUSION

The study suggest that, production of ROS is triggered by metabolism of naphthalene derivatives (naphthoquinone), accompanied by reduced oxidant/antioxidant balance that leads to a state of oxidative-stress. In this study, the oxidative stress is responsible for the hepatotoxicity and the disturbance in the level of hepatic enzymes. Therefore, a possible conclusion that such biochemical changes observed in these experimental animals may be seen in human beings is undeniable so naphthalene derivatives (naphthoquinone) have more toxic effects than therapeutic effects.

Conflict of interest: Authors declares no conflict of interest. No external funding was done.

REFERENCES

1. Goetz MP, Toft D, Ames M, Erlichman C. 2003. The Hsp 90 chaperone complex as a novel target for cancer therapy. Annals of oncology 14: 1169-1176
2. Aebi H. 1984. [13] Catalase in vitro. Pages 121-126. Methods in enzymology, vol. 105 Elsevier.
3. Ajani JA. 2008. Optimizing docetaxel chemotherapy in patients with cancer of the gastric and gastroesophageal junction: evolution of the docetaxel, cisplatin, and 5- fluorouracil regimen. Cancer 113: 945-955.
4. Bagchi D, Stohs SJ, Downs BW, Bagchi M, Preuss HG. 2002a. Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicology 180: 5-22.
5. Bagchi D, Balmoori J, Bagchi M, Ye X, Williams CB, Stohs SJ. 2002b. Comparative effects of TCDD, endrin, naphthalene and chromium (VI) on oxidative stress and tissue damage in the liver and brain tissues of mice. Toxicology 175: 73-82.
6. Barouki R, Morel Y. 2001. Repression of cytochrome P450 1A1 gene expression by oxidative stress: mechanisms and biological implications. Biochemical pharmacology 61: 511-516.
7. Buege JA, Aust SD. 1978. [30] Microsomal lipid peroxidation. Pages 302-310. Methods in enzymology, vol. 52 Elsevier.
8. Cerutti PA. 1985. Prooxidant states and tumor promotion. Science 227: 375-381.
9. Di Yang M, Shen XB, Hu YS, Chen YY, Liu XH. 2019. Novel naphthalene-enoates: Design and anticancer activity through regulation cell autophagy. Biomedicine & Pharmacotherapy 113: 108747.
10. Espinosa E, Zamora P, Feliu J, Barón MG. 2003. Classification of anticancer drugs—a new system based on therapeutic targets. Cancer treatment reviews 29: 515-523.
11. Fantini M, Benvenuto M, Masuelli L, Frajese GV, Tresoldi I, Modesti A, Bei R. 2015. In vitro and in vivo antitumoral effects of combinations of polyphenols, or polyphenols and anticancer drugs: perspectives on cancer treatment. International journal of molecular sciences 16: 9236-9282.
12. Kakkar R, Kalra J, Mantha SV, Prasad K. 1995. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. Molecular and cellular biochemistry 151: 113-119.
13. Mencarelli C, Martinez–Martinez P. 2013. Ceramide function in the brain: when a slight tilt is enough. Cellular and Molecular Life Sciences 70: 181-203.
14. Plopper CG, Van Winkle LS, Fanucchi MV, Malburg SR, Nishio SJ, Chang A, Buckpitt AR. 2001. Early events in naphthalene-induced acute clara cell toxicity: II. Comparison of glutathione depletion and histopathology by airway location. American journal of respiratory cell and molecular biology 24: 272-281.
15. Preuss R, Angerer J, Drexler H. 2003. Naphthalene—an environmental and occupational toxicant. International archives of occupational and environmental health 76: 556-576.
16. Qiu HY, Wang PF, Lin HY, Tang CY, Zhu HL, Yang YH. 2018. Naphthoquinones: A continuing source for discovery of therapeutic antineoplastic agents. Chemical biology & drug design 91: 681-690.
17. Rahman I, Kode A, Biswas SK. 2006. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nature protocols 1: 3159.
18. Ray SD, Kumar MA, Bagchi D. 1999. A novel proanthocyanidin IH636 grape seed extract increases in vivo Bcl-XL expression and prevents acetaminophen-induced programmed and unprogrammed cell death in mouse liver. Archives of biochemistry and biophysics 369: 42-58.
19. Revathi B, Prashanth K. 2015. Potential Hsp90 inhibitors: A novel target for cancer therapy. Chemotherapy 4: 2.
20. Seyfried TN, Shelton LM. 2010. Cancer as a metabolic disease. Nutrition & metabolism 7: 7.
21. Shewach DS, Kuchta RD. 2009. Introduction to cancer chemotherapeutics: ACS Publications.
22. Tamaki T, Naomoto Y, Kimura S, Kawashima R, Shirakawa Y, Shigemitsu K, Yamatsuji T, Haisa M, Gunduz M, Tanaka N. 2003. Apoptosis in normal tissues induced by anti-cancer drugs. Journal of international medical research 31: 6-16.
23. West JA, Pakehham G, Morin D, Fleschner CA, Buckpitt AR, Plopper CG. 2001. Inhaled naphthalene causes dose dependent Clara cell cytotoxicity in mice but not in rats. Toxicology and applied pharmacology 173: 114-119.
24. Weydert CJ, Cullen JJ. 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nature protocols 5: 51.
25. Yuan J-N, Chao Y, Lee W-P, Li C-P, Lee R-C, Chang F-Y, Yen S-H, Lee S-D, Whang-Peng J. 2008. Chemotherapy with etoposide, doxorubicin, cisplatin, 5-fluorouracil, and leucovorin for patients with advanced hepatocellular carcinoma. Medical oncology 25: 201-206