ORIGINAL ARTICLE

Modulation of Oxidative Status under Naphthalene Induced Nephrotoxicity in Mice

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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 along chemotherapy to overcome these untoward effects.
Aim: To investigate the nephrotoxic effect of intraperitoneal administration of naphthalene derivatives in mice.
Methods: Naphthalene (NA) derivative (Doxorubicin) was dissolved in Dimethyl sulfoxide (DMSO) and given to 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. 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 Urea, Creatinine, Albumin, Cholesterol and triglycerides were done by kit method, histological analysis was done.
Results: Naphthalene derivative toxicity was observed as the result showed that mean serum Urea and Creatinine level were higher in mice administered naphthalene derivative as compared to control group. In addition, histological changes such as glomerular congestion, focal tubular dilation with cast cell, shrinkage of renal glomeruli and completely obliterated Bowman's spaces were seen in kidney on different doses
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 nephrotoxicity and the disturbance in the renal biomarkers 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: Nephrotoxicity, Oxidative Stress, Antioxidants, Naphthalene, Naphthoquinone, DMSO

INTRODUCTION

Cancer is an advanced disease includes variety of changes in cellular physiology that eventually causes malignancies. Excessive and abnormal growth of cell (neoplasia) is the biotic end point for the disease. The past decades have showed that cancer is treatable with the help of chemotherapy, radiotherapy and surgery. These methods can be used alone or in combination causing important affects in cancer cell growth. Combination therapy and early diagnosis can improve survival(Shewach and Kuchta 2009).

In chemotherapy chemical agents are used to stop the growth and eradicate the tumor cells not only the primary tumor but the distant sites also, but it cannot difference between normal and the tumor cells. Naphthoquinone such as doxorubicin and Daunorubicin have been used in treating variety of cancers. Despite that they produce satisfactory effect in chemotherapy, they also reflect acute toxicity and adverse products(El-Sayyad et al. 2009). Anticancer drugs are classified into chemotherapy, immunotherapy and hormonal therapy. Chemotherapy includes various subtypes according to their mode of action: antibiotics, mitosis inhibitors, antimetabolites, alkylating agents, topoisomerase I and II inhibitors. Naphthalene is a doubled ring aromatic hydrocarbon alloy. Naphthalene derivatives (Naphthoquinones) exert insecticidal, microbial and wound healing effects as well as promising agent in cancer therapies(Qiu et al. 2018). Naphthoquinones are novel attraction for anticancer treatments, but due to their possible side effects and toxicity, the potential use is limited(Di Yang et al. 2019). 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. Excessive production of ROS induce damage such as gene mutations, cell death, chromosomal breakage and carcinogenesis(Cerutti 1985).

The concentration of ROS within the cell is dependent on the production and removal of these species by the antioxidant system. Antioxidants are present in large quantities within the cell that protect or restore the destruction produced by ROS. These enzymes include superoxide dismutase (SOD), catalase and glutathione peroxidase(Weydert and Cullen 2010).

Aim of this study was to evaluate the toxic effects of naphthoquinone used as anticancer drugs to normal cells of mice. Rather they have anticancer properties but are more vulnerable to the normal cells.

MATERIALS AND METHODS

Experimental work: The study was governed at Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore. The University of Lahore Animal Care and Use Council had approved all experimental protocols and animal procedures.

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Drug preparation: Naphthoquinone was dissolved in DMSO and a stock solution of 50mg/ml was made. Doses increase in preferable volume of maximum 0.5ml intraperitoneally in mice.

Animal and environmental conditions: White albino mice (female and male weighing 20-35 grams) were purchased out from the University of Lahore Animal House. Animals were put up in wide airy cages in groups 5 mice per cage along full access of water and food under controlled condition of temperature 25C and normal photoperiod (12 hours' dark and light) 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 checked for any toxic symptoms for early 2 hours and then at 6^{th} and 24^{th} hour. 24 hours after the injection number of expired mice were computed in each group. In animals acquiring intraperitoneal injection, abdominal muscle contraction, ataxia, altered breathing and heartrate pattern and itching was persistently observed for few hours. Drowsiness and diminish response was noticed post 6 hours. The severe effects were related to the high level of dose. However, post 24 hours of injection most of the survivor's symptoms were recovered, but alopecia was observed in the animals with high dose. LD50 of drug mixture was found to be 200 mg/kg by the number of mice died at each dose.

Study design: Experimental type of study was conducted using animal model. 15 adult male and female albino mice 8 weeks were obtained and randomly subdivided into three groups, five mice per group (n=5). The weight variation was limited to 20% of mean weight of animal before assigning groups. Group A (G-A), control group includes mice with no drug; Group B (G-B) mice were injected with i.p 50 mg/kg of drug mixture; Group C (G-C) comprises with i.p 150 mg/kg injection of drug mixture.

The maximum tolerated dose (MTD) is defined as the highest tolerated dose that will not produce any major toxicity which could be life threatening. After intraperitoneal injection of drug mixture on selected doses, mice were observed for 24 hours. All the animals survived 24h after the injections. There was a neglect able decrease in the body weight before and after the treatment. . The animals were weighed, anesthetized with chloroform, necropsied, and discarded after collection of all organs and blood samples to evaluate the histological, hematological and biochemical specifications. Animals were weighed before and at the end of the treatment. Blood samples directly from heart of all mice were collected in syringes for evaluation of renal function test (RFTs), lipid profile. Kidney were removed and after weighing organs from each mouse were divided into two halves, one half was preserved in 10%buffered formalin for histological analysis by light microscopy and one half was preserved at -80 C in separate falcon tubes for oxidative stress tests.

Table 1: Group designation and dose level

Groups	Doses
A	Control
B	Naphthalene @ 50 mg/kg/bwt i.p
C	Naphthalene @ 150 mg/kg/bwt i.p

Method: Mice were splited within three groups comprises of five animals respectively. Group A was treated as control. Group B and C were treated with Naphthoquinone 50 mg/kg/bwt i.p and 150mg/kg/bwt i.p for 30 days. The serum was obtained after centrifugation at 5000rpm for 5 min at 4C. Elisa kit method was used to assess serum creatinine, urea and albumin. Serum cholesterol and triglycerides were assessed to determine the lipid profile. For histopathological examination 10% formalin was used to preserve tissues by slicing (5micrometer thick) slices and imbedding them in paraffin blocks. Microscopic imaging was done after Hematoxylin-eosin staining(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 phosphate buffer solution 0.5 M having 7.2pH after that centrifugation done for 10 minutes each at 5000rpm. Further testing was performed after obtaining clear supernatant. Superoxide dismutase (SOD) action was computed by the method of Kakkar(Kakkar et al. 1995). MDA was estimated by using Beuge and Aust method(Buege and Aust 1978). The method of Aebi was used for measure catalase activity(Aebi 1984). Spectrophotometric/microplate reader assay method was used to evaluate glutathione (GSH) activity (Rahman et al. 2006).

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 statistical significance was $p< 0.05$. The correlation was calculated between the groups.

RESULTS

Naphthoquinone have highly significantly effect on renal and stress profile. The administration of naphthoquinone 50 and 150 mg/kg/bwt in mice leads to significant increase of biochemical marker. Higher levels of creatinine, urea and albumin were reported in both groups as compared to control and representing acute nephrotoxicity and kidney damage. The administration of naphthoquinone 50-150 mg/kg/bwt i.p in mice induced increase MDA levels was reported in group B (4.019±9.14) and C (7.19±1.14) as compared to control group A (0.89±0.011). Naphthoquinones have a deleterious effect on ant oxidative status. The lowest value of SOD, GSH and CAT in mice was reported in group C (0.17±0.0042) (5.19±1.08) and (9.18±2.16) respectively receiving naphthalene at the doses of 150 mg/kg/bwt i.p and has a significant difference with control group (0.78v0.004, 9.29±3.29 and 31.29±4.16) respectively. Moreover naphthoquinone have significant deleterious effects on the kidney as shown in table 02 levels of creatinine, urea and albumin were increased significantly (p=0.037, 0.024 and 0.015 respectively) in

control group C (0.775±0.12, 33.75±8.18 and 4.45±0.68 respectively) as compared to group B (0.725±0.15, 28.5±9.0 and 3.925±0.4717 respectively) and group A (0.65±0.1, 23.5±3.41 and 3.65±0.52) respectively.

Section from control group showing normal appearance of glomerulus. H&E stained sections from naphthoquinone 50 mg/kg group B showing shrinkage of glomeruli, presence of some cast cells seen, obliterated bowman's spaces, congestion of glomeruli and mesangial expansion also seen. H&E stained 150 mg/kg group C showing absent glomerulus capillary tufts with widened bowman's spaces and interstitial hemorrhage, complete obliteration in parenchyma with dispersed inflammatory cells.

Table 2: Biochemical variables of medical importance in mice under naphthalene induced renal insult

Groups	**Creatinine**	**Urea**	**Albumin**
A	0.65±0.01	23.5±3.41	3.65±0.12
B	0.834±0.02	28.5±2.4	3.925±0.1
C	0.926±0.24	33.75±1.8	4.45±0.24
P values	0.037	0.024	0.015

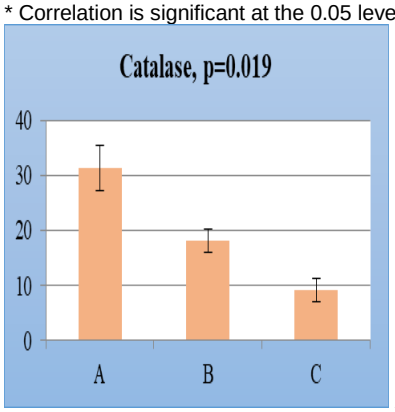
Table 3: Biochemical variables of oxidative stress in mice under naphthalene induced renal insult

Groups	Catalase	MDA	SOD	GSH
A	31.29±4.16a	0.89±0.011c	0.78±0.004a	9.29±3.29a
B	18.16±2.19b	9.14±4.019b	0.326±0.017c	6.29±2.33b
C	9.18±2.16c	7.19±1.14a	0.17±0.0042b	5.19±1.08b
P values	0.019*	0.032**	0.000***	0.034**

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

	Creatinine	**Urea**	**Albumin**	**GSH**	**SOD**	**MDA**	**Catalase**
Creatinine	1.000	.564 .021	.726 .000	-.681 .023	-.661 .011	.562 .011	-.519 .017
Urea		1.000	.652 .014	-.486 .185	-.598 .032	.618 .046	-.662 .016
Albumin			1.000	-.596 .025	-.745 .024	.745 .011	-.562 .236
GSH				1.000	.652 .032	-.645 .024	.532 .026
SOD					1.000	-.648 .000	.542 .024
MDA						1.000	-.459 .124
Catalase							1.000

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



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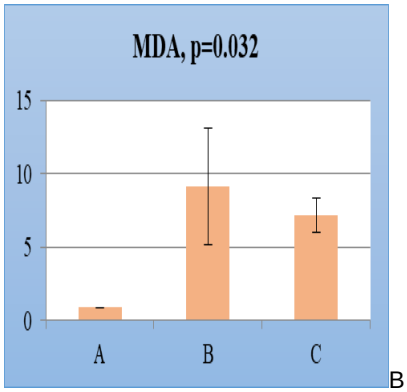

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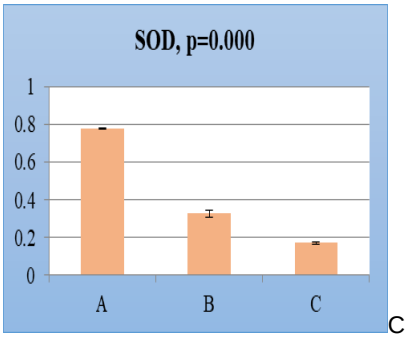

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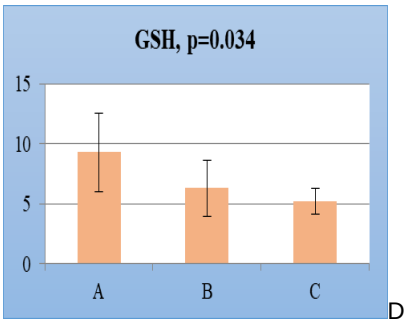

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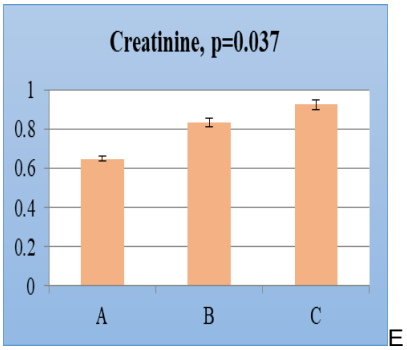

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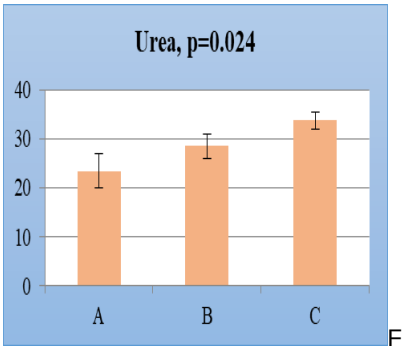

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Figure 1: Section from control group showing normal appearance of glomerulus (H&E, X400).

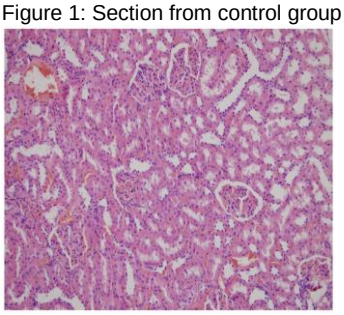


Figure 2: (a-c) H&E stained sections from naphthalene derivatives 50 mg/kg group B showing shrinkage of glomeruli, presence of some cast cells seen, obliterated bowman's spaces, congestion of glomeruli and mesangial expansion also seen.

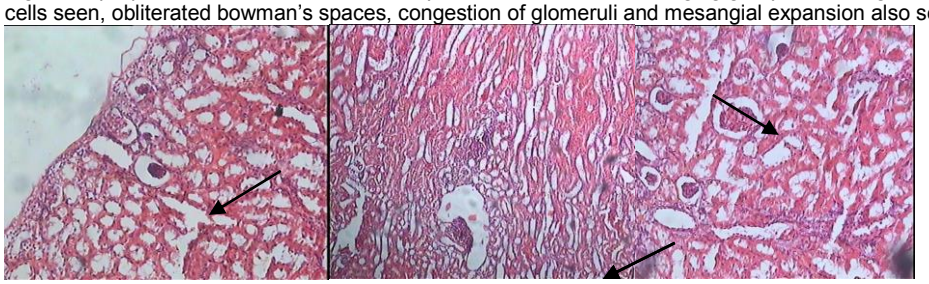


Figure 3: (a-c) H&E stained 150 mg/kg group C showing absent glomerulus capillary tufts with widened bowman's spaces and interstitial hemorrhage, complete obliteration in parenchyma with dispersed inflammatory cells.

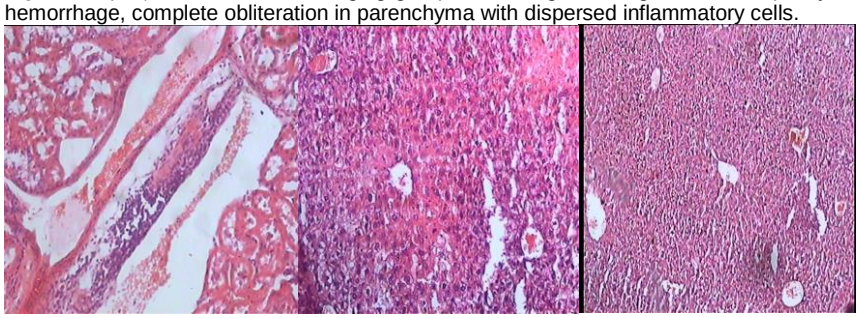
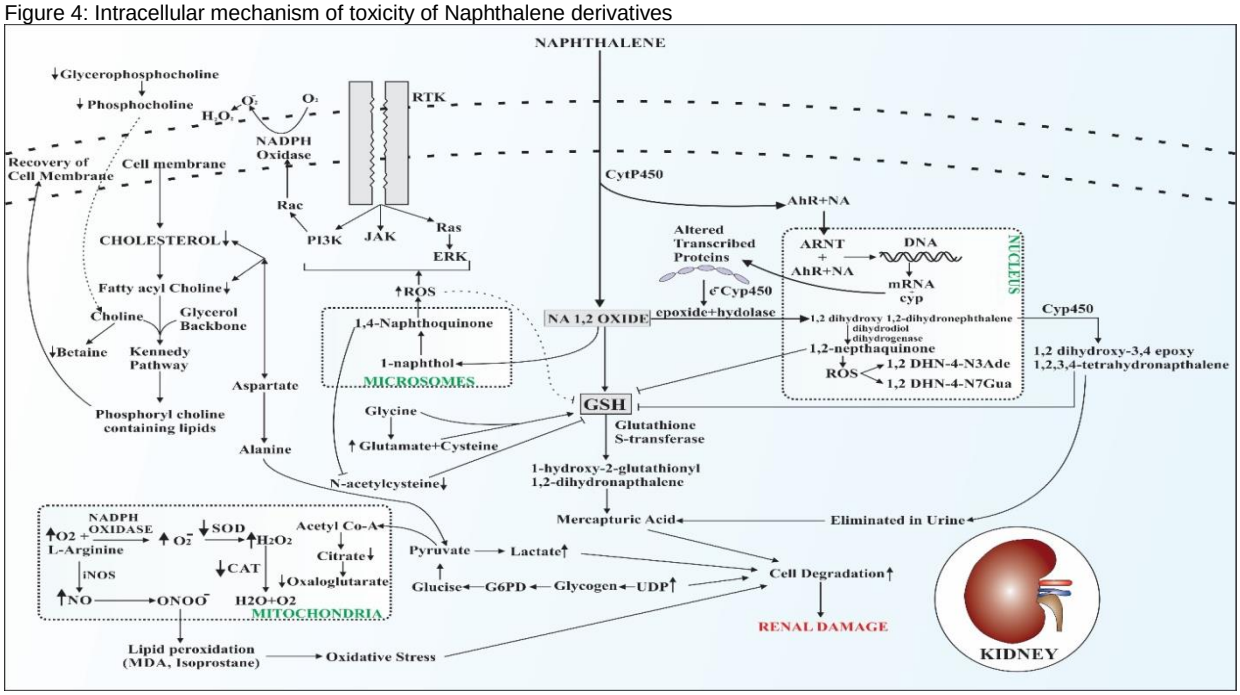


Figure 4: Intracellular mechanism of toxicity of Naphthalene derivatives



DISCUSSION

Cancer is a complex disease including many spatio temporal alterations in cellular physiology, which ultimately leads to malignancies. Tumor cell invasion to the nearby tissues and far by organs is the main cause of death in cancer patients. Anticancer drugs produce its effect at different stages: cancer cells, immune system or host cells, extracellular matrix and endothelium. Proteins, RNA and DNA of tumor cell are targets of drugs. Tumors DNA are the target of interest for many classical chemotherapeutic drugs. Specific antibodies and small molecules affect extracellular matrix and endothelium(Espinosa et al. 2003). Cancers are extensively been treated by chemotherapy such as doxorubicin, Cisplatin, and 5-FU(Yuan et al. 2008). However, they kill cancer cells, they also manifest nasty side effects and severe toxicity(Ajani 2008). Kidney is the essential organ that comprises the vital control system assisting the body homeostasis. It holds prominent regeneration ability. Many chemicals and drugs can affect it(Crawford et al. 2004). In vivo studies have shown that naphthalene causes cellular destruction and ultrastructural changes like cytoplasmic blabbing, intracellular glutathione depletion and swollen smooth endoplasmic reticulum(Plopper et al. 2001). Phospholipids are vital component of cell membrane act as an osmo-regulator and osmotic cell protectant found in animal cell membrane. They also play unique role in signal transduction(Kolesnick and Golde 1994). Cholesterol is vital structural component present in cell membrane which helps to maintain cell permeability and fluidity with intracellular transport and signaling. Naphthoquinone in high doses causes cholesterol elevation indicates excessive membrane disruption(Pörn et al. 1993). Parent compound of naphthalene are unable to cross glomerular filtration; whereas, reactive naphthalene adducts biotransformation by cytochrome P450s combines with glutathione and facilitation in excretion done by kidney in the form of mercapturic acid through urine. High level of mercapturic acid in urine indicates rapid metabolism and excretion of naphthalene after intraperitoneal injection(O'Brien et al. 1985). Mercapturic acid is largely excreted in urine as naphthalene metabolite and is suggestive of naphthalene induced toxicity(Willems et al. 2001). Naphthoquinone have strong potential to form free radicals. Rate limiting step in lipid peroxidation is free radical formation(Barouki and Morel 2001).

Our study shows elevations in mean serum urea, creatinine activities and albumin levels at different naphthoquinone concentration and that of histological changes are indicative of renal dysfunction. G-B animals treated with naphthoquinone 50mg/kg showed variable histological parenchymatous changes. Most of the glomeruli indicate glomerular congestion. Pronounced mesangial expansion seen in some glomeruli hence showed completely obliterated Bowman's spaces. Widening of Bowman's capsule with shrunken glomeruli also observed, dilated tubules with intratubular casts are seen. High-dose naphthalene derivatives 150 mg/kg treated group (G-C) animals kidney showed complete histological parenchymatous and tubular changes. Most of glomeruli showed absent glomerular capillary tufts, intra-glomerular hemorrhage also seen with widened bowman's spaces. Blood vessels congested in peritubular and intratubular region. Interstitial hemorrhage was seen with focal interstitial cellular infiltrates. The glomerular congestion and enlargement showed the structural and functional adaptation of the nephron. Focal segmental glomerulosclerosis is seen as hyper infiltration in nephron is its sign(Crawford et al. 2004). An imbalance between synthesis and degradation is a dynamic process that can result in the accumulation of matrix leading to progressive

glomerulosclerosis(Alderson et al. 2004). Atrophy of glomeruli and dilated capsular spaces describe shrunken glomerulus as sign of sclerosis. Tubulo-interstitial changes were seen in this study such as presence of cast cells suggests the presence of lipid peroxidation and free radical production. Lipid peroxidation describes the destruction of lipid and protein structure of intracellular membrane and hydrolyzes the cytoplasm(Saadi et al. 2008). Tubular cell death causes slugging off tubular lumen which contributes to cast formation. The casts obstruct the tubular lumen and increases tubular pressure, this causes leaking of ultra-filtrate across the tubular basement membrane(El-sherif and Issa 2015). Cellular infiltrates stated that NA provokes the inflammatory response leading to increased cytokines production and capillary permeability. Inflammatory response is initial defense mechanism but after that tissue damage starts to happen(Wu et al. 2005). The present result showed that naphthalene derivatives also have the same effect like naphthalene which makes it even more toxic in cancer cell treatment as it destroys the normal cells.

CONCLUSION

In this study production of ROS is triggered by naphthalene derivatives metabolism, leading to reduced oxidant/antioxidant balance, resulting in state of oxidative-stress that could have partially been accountable for the slight nephrotoxicity and the disturbance in the biomarker is seen in this study. Histological changes in the study suggest nephrotoxicity. Therefore, a possible conclusion that such biochemical changes observed in these experimental animals may be seen in human beings is undeniable so naphthoquinone have more toxic effects than therapeutic effects

Conflict of interest: Authors declares no conflict of interest. No external funding was done. All the research project expenses were born by authors themselves.

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