International Journal for Scientific Research (IJSR)

المجلة الدولية للبحوث العلمية

Vol. (4), No. (2) February 2025 الإصدار (4)، العدد (2)

The Role of Glutathione in Treating Pathological Changes Caused by Exposure to Phthalates in Some Tissues of Female Mice

Sarah I. Othman, Dima E. Baharoon*, Israa A. Kara, Heba H. Alkhalaf
Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia
*dimabaharoon@gmail.com

Islam A. El-redh, Alaa R. Khatab, Manal M. Almarii
Medical Research on Experimental Animals Administration, Central Research Laboratory, King Saud University, Riyadh 11451, Saudi Arabia

Abstract

Phthalates (PAEs) are the most widely used Polyvinyl chloride (PVC) plasticizers. They are produced with a global production of 470 million pounds. It is extensively used in many consumer products such as cosmetics, toys, bottles, food packaging, medical plastic devices, building and construction. It has become a serious problem in recent years. PAEs can be introduced particularly by dermal absorption, ingestion and air inhalation. Laboratory animal studies have reported the endocrine disrupting effects of PAEs and have been recognized as substances of high concern due to their property of reaching animals tissues and inducing a negative impact on health. Our current study involved oral injection of Potassium Hydrogen Phthalate and Glutathione as an antioxidant daily for 3 weeks to investigate the adverse effects of phthalate on the organ tissues (Ovary, liver and kidney) and improving tissue damage resulting from phthalates using the antioxidant glutathione in female Swiss mice. The results revealed organs damage associated with impaired oxidative stress and histopathological changes such as intravenous bleeding, cell destruction, chromatin

condensation and apoptosis in all organs. Treatment with glutathione restricts PAE negative impacts and showed histological improvements such as reappearance of organ's cells and minimized bleeding in all tested organs.

Keywords: Endocrine disruptors, Phthalates, Oxidative Stress, Antioxidants, Glutathione, Apoptosis, Congestion.

Introduction

Exposure to persistent pollutants (Phthalates) has been associated with the development of certain human diseases such as diabetes, cancer, induction alterations in puberty, and fertility disorders in both males and females. At the hormonal level, phthalates can modify the release of pituitary and hypothalamus hormone. At the intracellular level, phthalates have the ability to alter gene expression associated with reproduction and interfere with membrane receptors within cells (Hlisníková, et al.,2020).Also, with continuous exposure it may have detrimental effects on certain organs (Amara, et al.,2020).Phthalate is hazardous to people and the environment and are considered to be among the most important Endocrine-Disrupting Chemicals (EDCs) that are known to be risk factors. The World Health Organization (WHO) defined the endocrine disruptor chemical as "An exogenous substance or mixture that alters functions of the endocrine system and consequently causes adverse health effects in an intact organism", due to their widespread use in daily life, Phthalates have drawn a lot of attention from the scientific and medical community (Rolfo, et al.,2020). Furthermore, phthalates are plasticizers that have lipophilic properties that enable them to interact with lipid storage depots and membranes, which promote bioaccumulation and biomagnification along the food chain (AlSaleh, et al.,2017). Phthalates can leach from plastics into water, beverages, foods, and body fluid (Bhaisare, et al.,2022). Humans are exposed to over 18 billion pounds of phthalate yearly by food, inhalation, and skin absorption on a daily basis (Amara, et al.,2020). Solving this

increasingly serious problem requires an understanding of the mechanisms underlying the impacts of phthalates. Nevertheless, there isn't enough of this complicated knowledge at the moment. Therefore, we consider it essential to generate such a comprehensive overview of the topic. In this study, we made an effort to provide an overview of the most recent research on the impact of phthalates on the body organs and finding a treatment to reduce or prevent this effect (Hlisníková, et al.,2020). This study aims to determine the impact of phthalates and to examine the histological changes on Ovary, liver and kidneys. Also, to shed the spotlight on treating the adverse effects of phthalates by using an obtainable antioxidant.

Materials and Methods

The experimental procedures were performed and approved by Princess Nourah Bint Abdulrahman University Institutional Review Board (PNU-IRB) (ethical number: 0699).

Experimental animals:

Adult female Swiss mice were used in this experiment, which were obtained from the animal house of the Ceneral Research Laboratory at King Saud University in Riyadh. Animals were selected for this research with an average age of 12-13 weeks and an average body weight of 38-42 grams. The animals were distributed in special cages equipped with feeding bottles to drink water in a ventilated room subject to the appropriate natural factors of humidity, light, and temperature ranging between 25°C and 35°C.

Phthalate and glutathione:

Phthalates were used in the form of Potassium Hydrogen Phthalate (KHP) and were obtained from SATC (Saudi Trading & Technology Company) in the form of a white powder in a box weighing 500 grams Glutathione were obtained from the online retail company IHERB in the form of capsules containing 200 mg of powdered

glutathione. Phthalates & glutathione were dissolved separately in distilled water and by gavage the dose was given to the mice for a period of three weeks. For female mice, a dose of 200 mg/kg of body weight (Sudha, et al.,2013).

Animals and experimental design:

Experimental animals are divided into the following groups:

- **The first group:** Control group: Group of animals that are given distilled water and this group includes 5 female mice.
- **The second group:** Antioxidant Group: Group of animals that are given antioxidant and this group includes 5 female mice. The antioxidant glutathione is given by gavage at a dose of 200 mg/kg body weight (Sudha, et al.,2013) dissolved in distilled water for 3 weeks.
- **The third group:** Phthalate Group: Group of animals that are given potassium hydrogen phthalate, It includes 5 female mice, phthalate is given by gavage at a dose of 200 mg/kg body weight (Sudha, et al.,2013) dissolved in distilled water for 3 weeks.
- **The fourth group:** Phthalate and Glutathione Group: Group of animals include 5 female mice that are given phthalates by gavage at a dose of 200 mg / kg of body weight dissolved in distilled water and then after an hour of taking the dose the mice are given the antioxidant glutathione at a dose of 200 mg / kg of body weight (Sudha, et al.,2013) dissolved in distilled water for 3 weeks.

Histological examination:

Histological examination of (liver, kidney and ovaries), tissues were sectioned and then stained with Hematoxylin and Eosin stain (H & E) and observed under the microscope.

International Journal for Scientific Research (IJSR)
المجلة الدولية للبحوث العلمية
Vol. (4), No. (2) February 2025 الإصدار (4)، العدد (2)

Observation and Results Histological changes in ovary

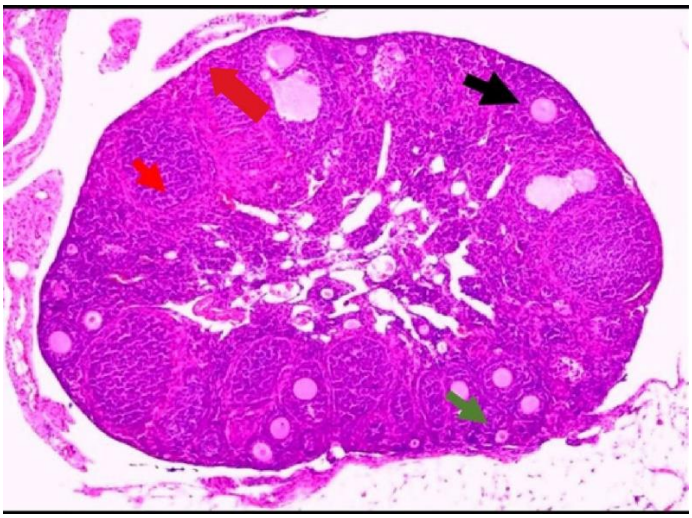


Fig.1(a): Photomicrograph of a section of control mice ovary showing a mature Graafian Follicle with ovum and its nucleus and nucleolus showed in (Black arrow), Also seen Germinal Epithelium (GE) (Red Arrow) and Primary Follicle (PF) showed in (Green Arrow) image indicate normal size and number. (H & E stain x400).

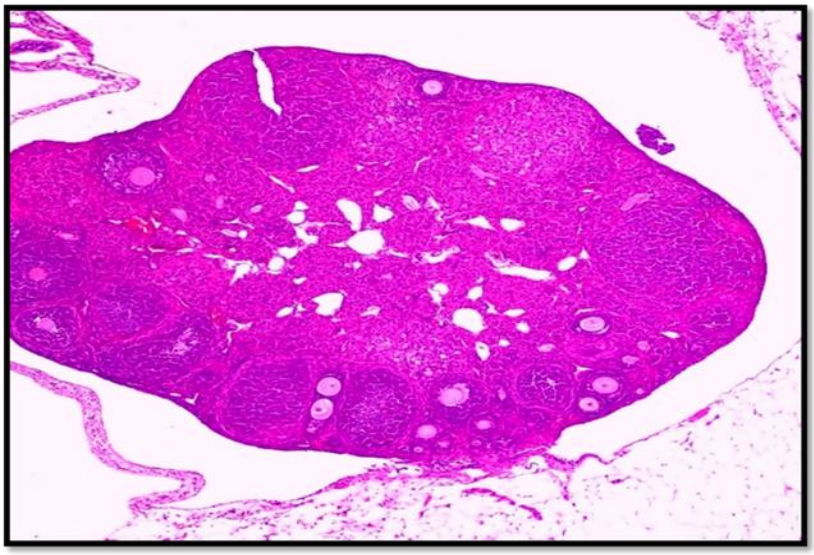
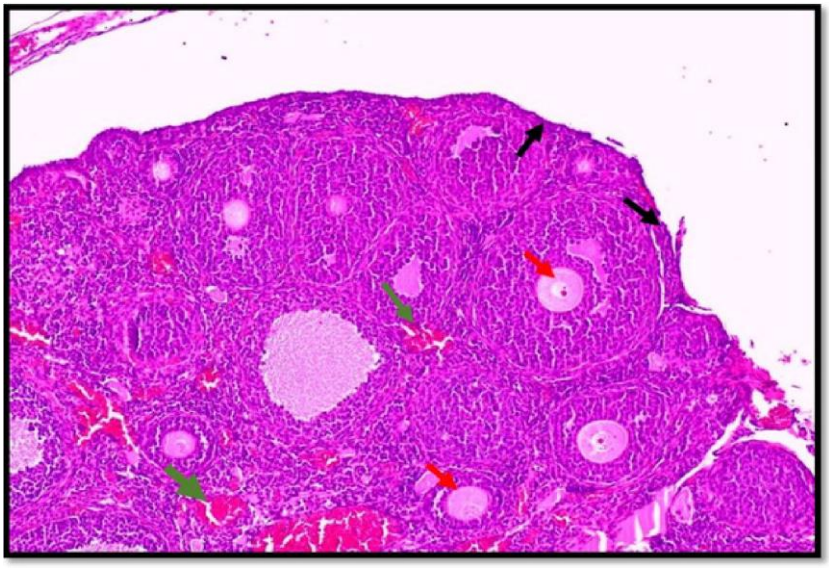
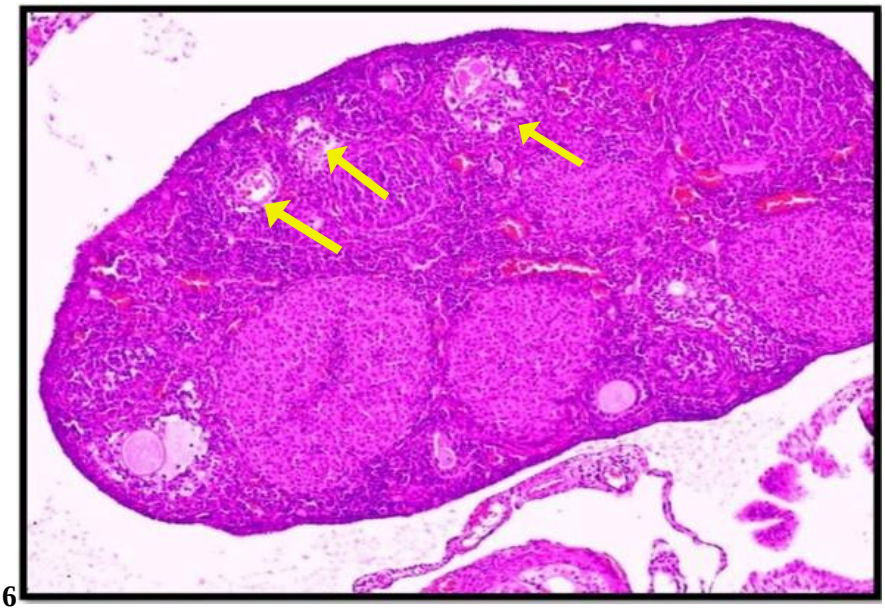


Fig. 1(b): Mice ovary of glutathione group (H and E stain: x400)





6

Fig.1(c, d): Mice ovary of phthalates group showed increase in the thickness of Tunica Albuginea (Black arrow), Corpus Luteum (CL) (white arrow), Congestion and Atretic Follicles (AF) (red arrow) and Intravenous bleeding (Green arrow). Also, cell death (Apoptosis) showed in (yellow arrow). (H & E stain: x400)

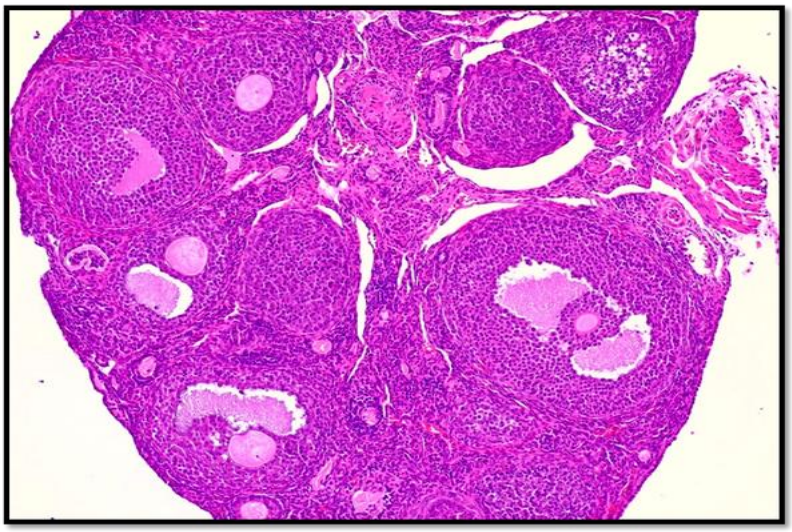


Fig.1(e): Mice ovary of phthalates and glutathione group: significant improvement in ovary tissues with no evidence of congestion. (H and E stain: x400)

Observation and Results Histological changes in Liver

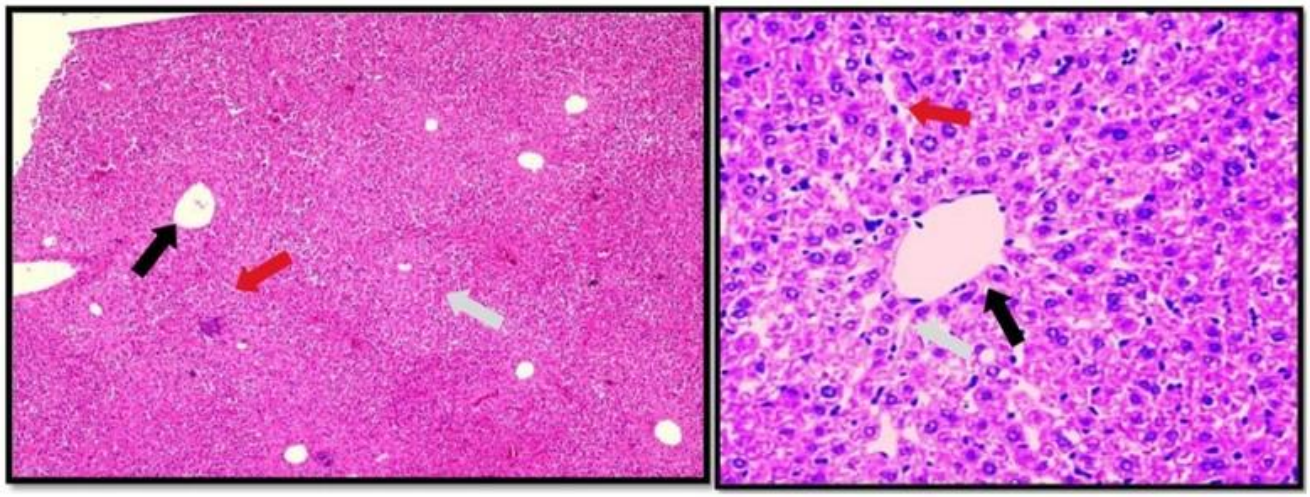


Fig. 2(a-b): Mice liver of control group, (a-b) Normal structure of the liver tissues with normal central vein (CV) (black arrows) surrounded by cords of hepatic cells (white arrows) and sinusoids (S) (red arrows) (H and E stain: x400).

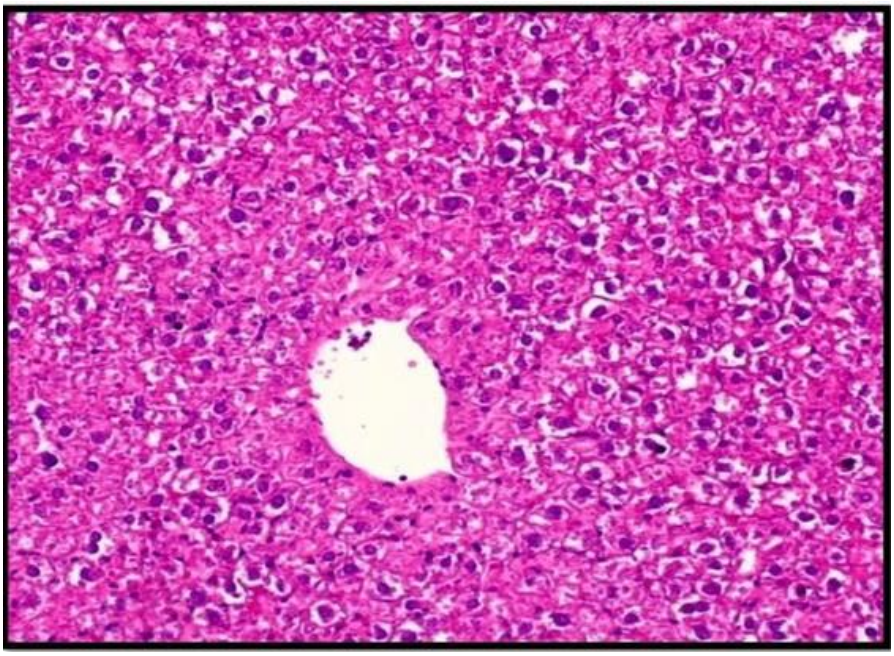


Fig. 2(c): Mice Liver of glutathione group, normal liver tissues (H and E stain: x400)

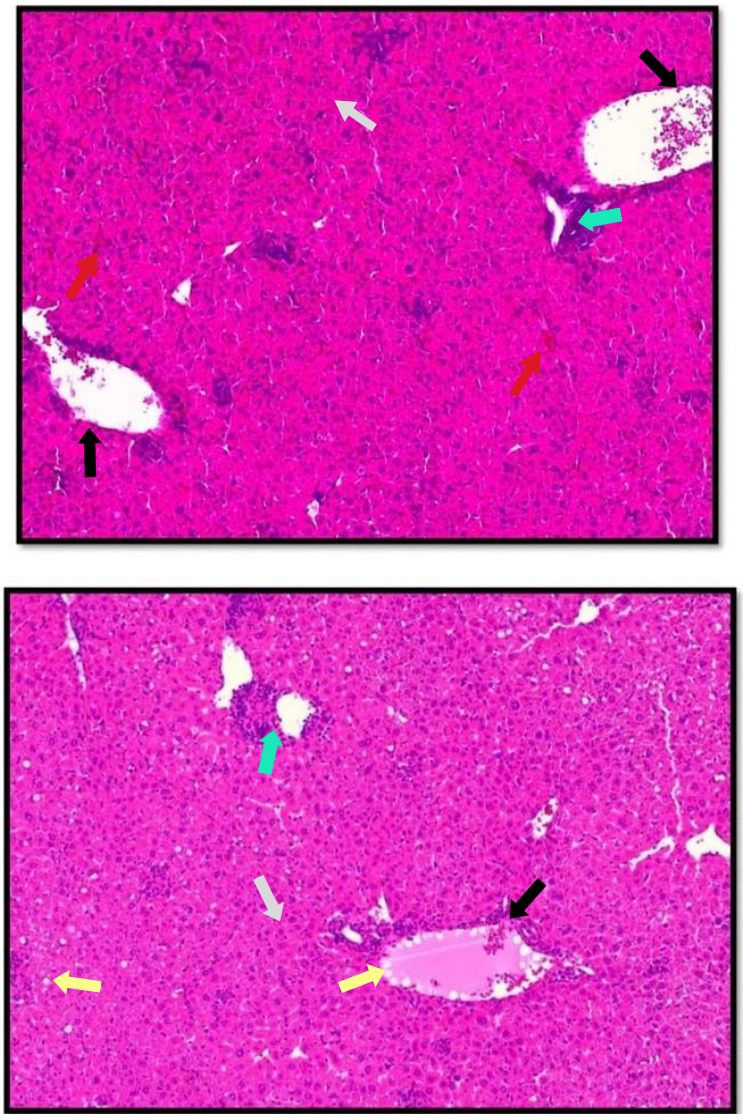


Fig.2 (d, e): Mice liver of phthalates group. Showed congestion and bleeding in central venous (black arrows) with death of most liver cells (white arrows), invasion of inflammatory cells and infiltrations around the C.V (green arrows), bleeding and enlargement of the hepatic sinusoids (red arrows). Also, presence of lipid droplets in the hepatocytes (yellow arrows) (H and E stain:x400).

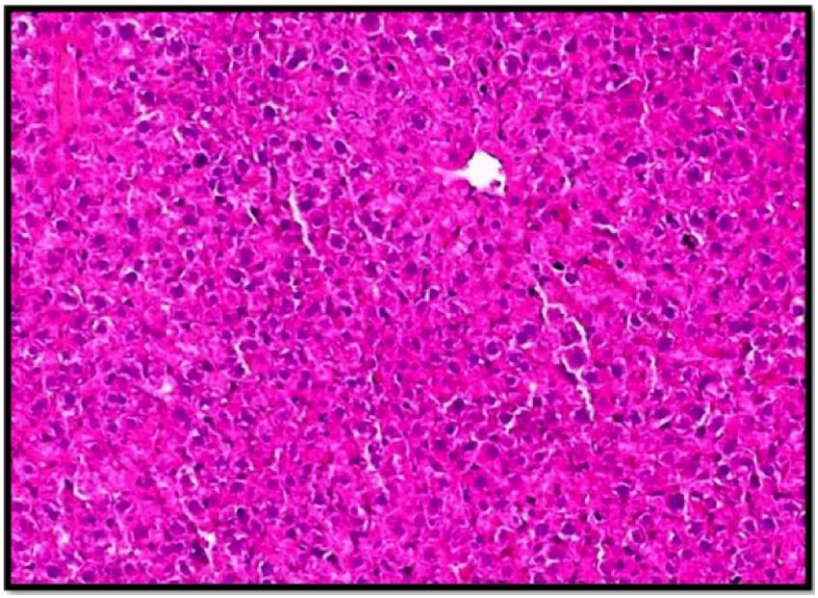


Fig. 2 (f): Mice liver of phthalates and glutathione group, significant improvement in liver tissue (H and E stain: x400). some areas around the central vein (Fig. 2(d, e). [Fig. 2(f)) Treatment with glutathione resulted in a significant improvement in liver tissue.

International Journal for Scientific Research (IJSR)
المجلة الدولية للبحوث العلمية
Vol. (4), No. (2) February 2025 الإصدار (4)، العدد (2)

Observation and Results Histological changes in Kidney

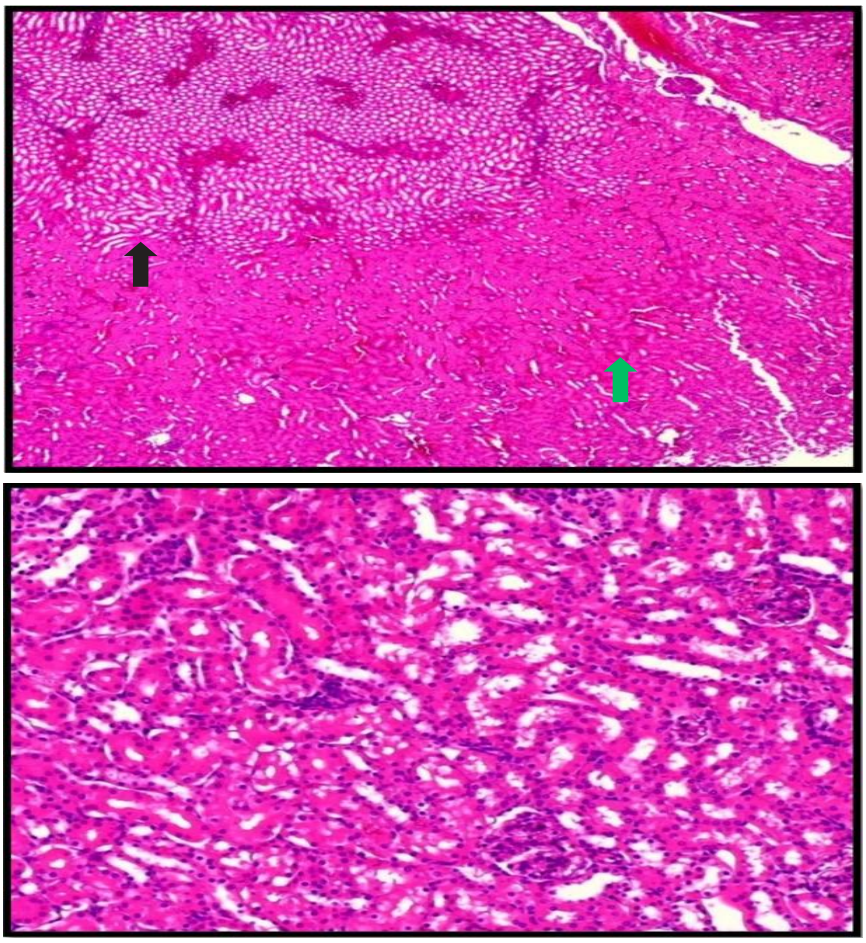


fig.3 (a,b): Mice Kidney of control group. In normal tissue, showed normal renal cortex (green arrow), a medullary ray which are a collection of renal tubules (proximal and distant convoluted) that drain into a single collecting duct (black arrow) (H and E stain:x400**).**

International Journal for Scientific Research, London Vol (4), No (2), 2025
https://doi.org/10.59992/IJSR.2025.v4n2p10 E-ISSN 2755-3418

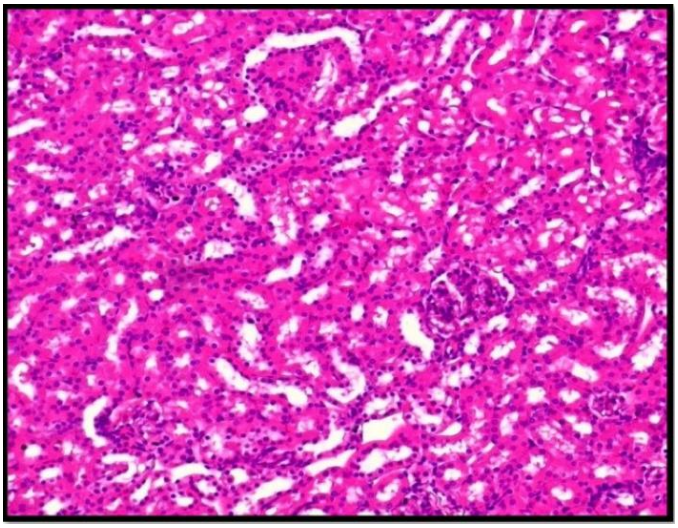
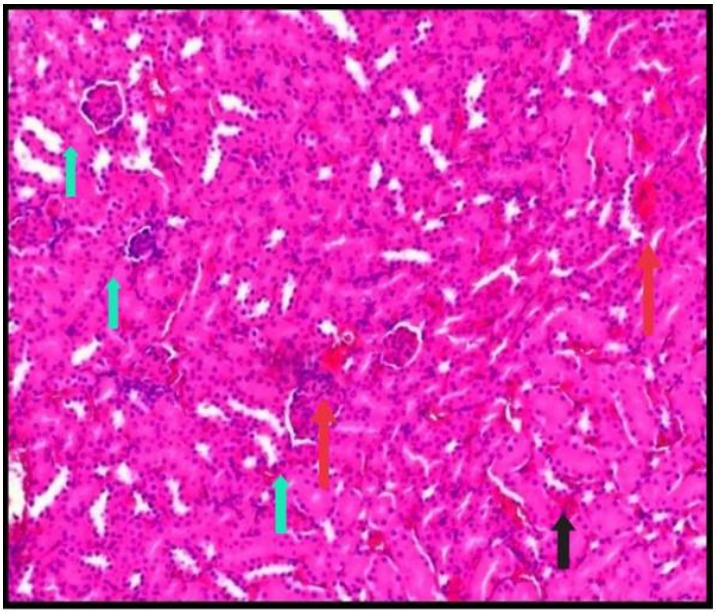


Fig. 3 (c): Mice kidney glutathione group. normal kidney tissues (H and E stain: x400)



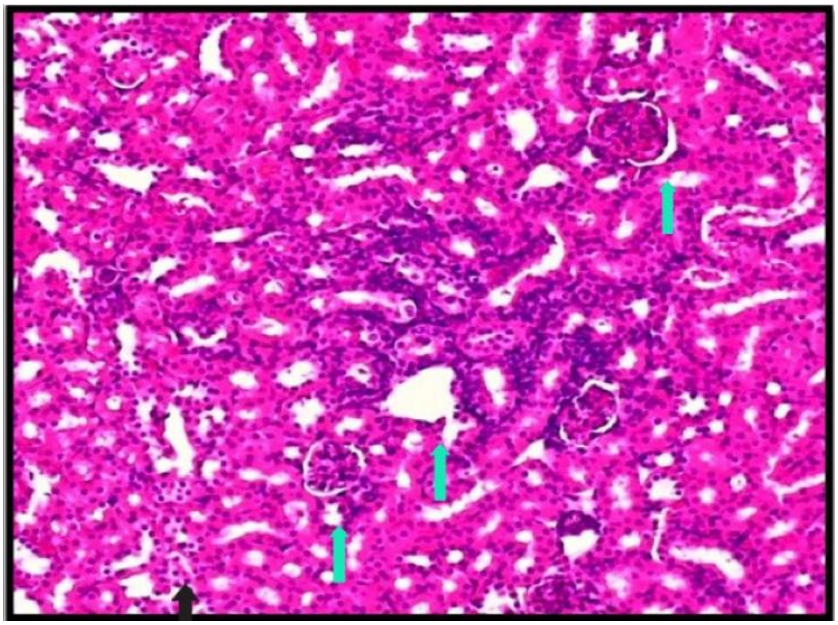


Fig. 3(d,e): Mice kidney phthalates group. kidney tissues showed visible bleeding in some regions of renal tissue (red arrow), shrinkage and destruction and disappearing (cell death) of the glomerulus (green arrow), severe congestion and destruction of the walls of renal tubules and chromatin condensation resulting death in most cells (black arrow) (H and E stain: x400).

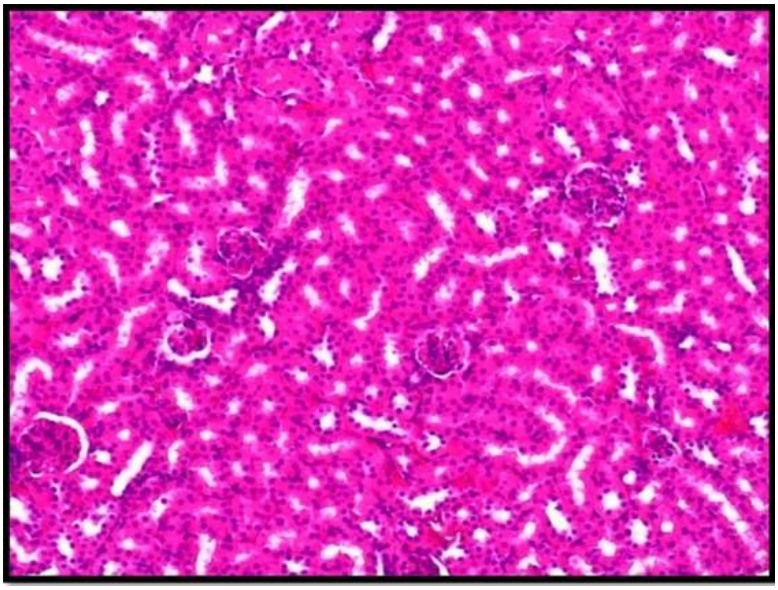


Fig.3(f) : Mice kidney phthalates and glutathione group .significant improvement in kidney tissue. (H and E stain: x400)

Discussion

The results of this systematic review provide robust evidence that phthalates are toxic and dangerous substance that leads to serious diseases such as cancer and diabetes, as well as oxidative stress damage and changes in the tissues of organs such as the ovary, liver and kidney. In our study there was a noticeable effect of phthalates on ovary tissues represented by the appearance of many atretic follicles and congestion, in addition to the cell apoptosis represented by programmed cell death and intravenous bleeding in many regions. Also, there was a decrease in the number of follicles compared to normal tissue. Our results were consistent with many studies in which the ovaries were treated with different amounts and periods of phthalates, as the results of previous studies showed distribution of ovarian follicles, disintegrated nucleus and fused and disintegrated oocyte (Bhaisare, et al.,2022). In addition cellular degeneration and angiectasia, atretic follicles, cystic follicle, vascular proliferation and anovulation that indicated by the absence of the corpus

luteum (Jebara,et al.,2022). Moreover, disappearance of the oocyte in the primary follicle, separation of granular cells, cell death, mitochondrial enlargement and cytoplasm vacuolation (Qin, et al.,2019).

Also, there was a noticeable effect of phthalates on liver tissues. It showed bleeding and congestion in central venous with death of most liver cells, invasion of inflammatory cells around and infiltrations around the C.V, bleeding and enlargement of the hepatic sinusoids. Also, presence of lipid droplets in the hepatocytes. Our results were consistent with many studies in which the liver was treated with different amounts and periods of phthalates which noticed in several studies that phthalates cause oxidative stress and inflammation in the liver of organisms, leading to programmed cell death (Cui, et al.,2020). Exposure leads to impaired lipid metabolisms and lipid accumulation that contribute to liver toxicity (Aydemir, et al.,2023). Moreover, inhibition of liver detoxifying enzymes and increase in DNA replication in rat hepatocytes, which causes genomic instability and apoptosis suppression which lead to development of tumors. Also, considerable changes including the enlargement of hepatic sinusoids, vena centralis degeneration, vacuole formations, degeneration in hepatocytes, and presence of lipid droplets in the hepatocytes (Praveena, et al.,2018). Our results were consistent with many studies in which the kidney was treated with different amounts and periods of phthalate the kidney tissue showed a serve destruction was observed which resulted death and decomposition of most cells, Moreover to an intensification of the nuclear chromatin and the shrinkage and disappearance in renal globules with the appearance of hemorrhage and narrowness within the glomerular cavity , Also blood congestion and destruction of the walls of renal tubules resulting large areas of destruction and visible bleeding leaving only the remains of some cells. It has been proven that one of the main ways phthalate metabolites are eliminated is through urinary excretion, so the kidney can be considered as a target organ. Following oral administration

phthalates mostly concentrate in the kidneys where their metabolites are known to trigger the activation of genes linked to oxidative stress (Erkekoglu & Kocer-Gumusel,2014).

Mice kidneys had the highest detectable concentrations of DEHP, which can lead to a marked decline in kidney function as measured by creatinine clearance (Miura, et al.,2007). In the kidney of quail, glomerular contraction and dilatation of renal tubular epithelial cells were seen. Female quail were fed a diet containing 0, 250, 500, and 750 mg/kg via gavage administration for 45 days (Li, et al.,2018).Environmental exposure to phthalates during daily life can have serious consequences on kidney function and contribute with progressive kidney damage over a lifetime affecting glomerular filtration rate, blood pressure and the concentration of uric acid in serum (Kataria, et al.,2015). Therefore we used the antioxidants to treat those adverse effects of phthalates and we chose glutathione as it is one of the most significant antioxidants that produced naturally by the liver and consists of three building blocks of protein or amino acids- glycine, cysteine and glutamine (Adeoye, et al.,2018). Also, play critical roles in neutralization of free radicals which produced by phthalates (Pizzorno,2014).

We noticed in our study that the group treated with phthalates and glutathione had a significant improvement in the organs, as in mice ovary glutathione repaired the atretic follicles and minimized bleeding in several places. Also, stopped cell destruction and cell death, reduced congestion and restored the normal size of ovarian follicles. Our results were consistent with many studies in which the ovaries were treated with different amounts of glutathione. The results of previous studies showed that the glycine, one of the main components of glutathione (GSH) promoted cumulus cell expansion and oocyte maturation and decreased the ROS production levels of oocytes. So, they use it to protect porcine oocytes in vitro (Gao, et al.,2023). Also, glutathione shields eggs from damage caused by oxidative stress during

folliculogenesis. Thus, GSH works to protect ovarian follicles (Adeoye, et al.,2018). In agreement with the results of previous studies, glutathione demonstrated its strong effect on improving ovarian tissue. We observed in our study that the group treated with phthalates and glutathione had a significant improvement in liver which reduce the bleeding and decrease in apoptosis. Also, in previous studies on the affect of glutathione on liver injures induced by other chemicals showed ameliorating in the hepatocellular damage in rats and had a protective effect on liver injuries (Jiang, et al.,2010). In other study on liver injures induced by chemical, glutathione increased free radical elimination and exerted protective effect by attenuating the release of inflammatory mediators (Lu, et al.,2010). Our study was consistent with previous studies. In addition, we noticed in our study that the group treated with phthalates and glutathione had a significant improvement in kidneys, GSH restricted phthalates activity in kidney by capturing the free radicals produced by phthalates and minimize the visible bleeding in some regions of renal tissues, Also GSH reduced cells destruction and cell apoptosis and helped in the reappearance of renal globules and the renal tubules. Moreover, with the similarity of previous studies GSH showed a noticeable improvement in kidney tissue by ameliorating patient's overall kidney functioning (Santos, et al.,2008). Another study mentioned that kidney damage (AKI) is being treated with RGSH, In a mouse model RGSH intervention decreased the degree of renal structural damage and ameliorated glomerular damage and improved cell viability, More results show that RGSH may inhibit cell apoptosis through the ferroptosis signaling pathway (He, et al.,2023).

Conclusion

From this study, we conclude that exposure to phthalates caused many damages to ovarian, liver, and kidney tissues. However, glutathione as antioxidant attenuated these adverse changes by minimizing the harmful effects of free radicals on cells and playing a notable role in antioxidation and detoxification of endogenous and

exogenous compounds. Therefore, these findings indicated that Glutathione is a potential antidote to alleviate and improve the exposed tissues to phthalates toxicity.

Recommendations

Through this study we recommend to Introduce the antioxidants into our diet and minimize sources of phthalate exposure by avoiding foods packaging and plastics, personal care products containing phthalates to protect body from toxins and to promote longevity. Also, encouraging companies to produce products that do not contain toxic chemicals and setting up some Guidelines to reduce exposure to EDCs. Furthermore, we recommend you to buy fresh or frozen fruits and vegetables instead of canned and processed versions. As well as using glass, stainless steel, ceramic, or wood to preserve and store foods. Finally, we recommend to conducting more researches and testing on treatment of phthalates and pay attention and shed the spotlight on EDCs as a global concern.

Acknowledgement

We would like to express our thanks and appreciation for the guidance and efforts to our supervisor Dr. Sarah Alothman, we also would like to give special thanks to the Advanced Cell Laboratory for their assistance with this project. The authors extend their appreciation to The Central Research Laboratory/ Research Institute/ Center Supporting Program (RICSP-25-3), King Saud University, Riyadh, Saudi Arabia. Furthermore, we are thankful to all our professors in the department for their contributions in our academic and personal growth. Finally, we would like to express our gratitude to our families and friends for being our constant source of support and encouragement throughout our academic journey.

References

- Adeoye, O., Olawumi, J., Opeyemi, A., & Christiania, O. (2018). Review on the role of glutathione on oxidative stress and infertility. JBRA assisted reproduction, 22(1), 61–66. https://doi.org/10.5935/1518-0557.20180003
- Al-Saleh, I., Elkhatib, R., Al-Rajoudi, T., & Al-Qudaihi, G. (2017). Assessing the concentration of phthalate esters (PAEs) and bisphenol A (BPA) and the genotoxic potential of treated wastewater (final effluent) in Saudi Arabia. The Science of the total environment, 578, 440–451. https://doi.org/10.1016/j.scitotenv.2016.10.207
- Amara, I., Salah, A., Timoumi, R., Annabi, E., Scuto, M., Trovato, A., Neffati, F., Calabrese, V., & Abid-Essefi, S. (2020). Effect of di(2-ethylhexyl) phthalate on Nrf2-regulated glutathione homeostasis in mouse kidney. Cell stress & chaperones, 25(6), 919–928. https://doi.org/10.1007/s12192-020-01127-8
- Aydemir, D., Aydogan-Ahbab, M., Barlas, N., & Ulusu, N. N. (2023). Effects of the in-utero dicyclohexyl phthalate and di-n-hexyl phthalate administration on the oxidative stress-induced histopathological changes in the rat liver tissue correlated with serum biochemistry and hematological parameters. Frontiers in endocrinology, 14, 1128202. https://doi.org/10.3389/fendo.2023.1128202
- Bhaisare, L. Y., Zade, S. B., Nagwanshi, A. M., Netam, A. K., Chaudhary, D. D. (2022). Impacts of Benzyl Butyl Phthalate on Histo-Architecture of Gonads of African Catfish Clarias gariepinus (Burchell, 1822). Polish Journal of Environmental Studies, 31(2). https://doi.org/10.15244/pjoes/142140
- Cui, Y., Zhang, Q., Yin, K., Song, N., Wang, B., & Lin, H. (2020). DEHP-induce damage in grass carp hepatocytes and the remedy of Eucalyptol. Ecotoxicology and environmental safety, 206, 111151.

  https://doi.org/10.1016/j.ecoenv.2020.111151
- Erkekoglu, P., & Kocer-Gumusel, B. (2014). Genotoxicity of phthalates. Toxicology mechanisms and methods, 24(9),616–626.

  https://doi.org/10.3109/15376516.2014.960987

- Gao, L., Zhang, C., Zheng, Y., Wu, D., Chen, X., Lan, H., Zheng, X., Wu, H., & Li, S. (2023). Glycine regulates lipid peroxidation promoting porcine oocyte maturation and early embryonic development. Journal of animal science, 101, skac425. https://doi.org/10.1093/jas/skac425
- He, L., & Shi, Y. (2023). Reduced glutathione ameliorates acute kidney injury by inhibiting ferroptosis. Molecular medicine reports, 27(6), 123.
- Hlisníková, H., Petrovičová, I., Kolena, B., Šidlovská, M., & Sirotkin, A. (2020). Effects and Mechanisms of Phthalates' Action on Reproductive Processes and Reproductive Health: A Literature Review. International journal of environmental research and public health, 17(18), 6811.

  https://doi.org/10.3390/ijerph17186811
- Jebara, A., Beltifa, A., Di Bella, G., Mabrouk, L., & Ben Mansour, H. (2022). Endocrine-disruptor endpoints in the ovary and thyroid of adult female rats exposed to realistic doses of di-(2-ethylhexyl) phthalate. Journal of water and health, 20(8), 1256–1267. https://doi.org/10.2166/wh.2022.140
- Jiang, N., Lu, L., Wang, T., Zhang, L., Xin, W., & Fu, F. (2010). Reduced glutathione attenuates liver injury induced by methyl parathion in rats.

  Toxicology mechanisms and methods, 20(2), 69–74.

  https://doi.org/10.3109/15376510903575782
- Kataria, A., Trasande, L., & Trachtman, H. (2015). The effects of environmental chemicals on renal function. Nature reviews. Nephrology, 11(10), 610–625. https://doi.org/10.1038/nrneph.2015.94
- Li, P. C., Li, X. N., Du, Z. H., Wang, H., Yu, Z. R., & Li, J. L. (2018). Di (2-ethyl hexyl) phthalate (DEHP)-induced kidney injury in quail (Coturnix japonica) via inhibiting HSF1/HSF3-dependent heat shock response. Chemosphere, 209, 981–988. https://doi.org/10.1016/j.chemosphere.2018.06.158
- Lu, L., Wang, X., Lang, L., & Fu, F. (2010). Protective effect of reduced glutathione on the liver injury induced by acute omethoate poisoning. Environmental toxicology and pharmacology, 30(3), 279–283.

https://doi.org/10.1016/j.etap.2010.08.001

- Miura, Y., Naito, M., Ablake, M., Terayama, H., Yi, S. Q., Qu, N., Cheng, L. X., Suna, S., Jitsunari, F., & Itoh, M. (2007). Short-term effects of di-(2-ethylhexyl) phthalate on testes, liver, kidneys and pancreas in mice. Asian journal of andrology, 9(2), 199–205. https://doi.org/10.1111/j.1745-7262.2007.00220.x
- Pizzorno J. (2014). Glutathione!. Integrative medicine (Encinitas, Calif.), 13(1), 8–12.
- Praveena, S. M., Teh, S. W., Rajendran, R. K., Kannan, N., Lin, C. C., Abdullah, R., & Kumar, S. (2018). Recent updates on phthalate exposure and human health: a special focus on liver toxicity and stem cell regeneration. Environmental science and pollution research international, 25(12), 11333– 11342. https://doi.org/10.1007/s11356-018-1652-8
- Qin, X. Y., Ma, Q., Xu, X. Y., Tan, Q., Zheng, K., & Wang, B. Y. (2019). Di-2Ethylhexyl Phthalate Induces Ovarian Toxicity and Alters Protein Expression of Hormone-regulated Receptors in Rats. Biomedical and environmental sciences: BES, 32(7), 546–549. https://doi.org/10.3967/bes2019.071
- Rolfo, A., Nuzzo, A. M., De Amicis, R., Moretti, L., Bertoli, S., & Leone, A. (2020). Fetal-Maternal Exposure to Endocrine Disruptors: Correlation with Diet Intake and Pregnancy Outcomes. Nutrients, 12(6), 1744. https://doi.org/10.3390/nu12061744
- Santos, N. A., Bezerra, C. S., Martins, N. M., Curti, C., Bianchi, M. L., & Santos, A. C. (2008). Hydroxyl radical scavenger ameliorates cisplatin-induced nephrotoxicity by preventing oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. *Cancer chemotherapy and pharmacology*, *61*(1), 145–155. https://doi.org/10.1007/s00280-007-0459-y
- Sudha, M., Rajkumar, D., & Felix, J. W. (2013). Protective effect of glutathione against isoproterenol induced myocardial injury in rats. Indian journal of physiology and pharmacology, 57(2), 132–137.