Antioxidant Effects of Resveratrol Against Cadmium Chloride in Albino Male Rabbits

Anas A. Humadi[1*], Raad J. Hammadi[2] and Ayat J. Mohammed[3]

[1]Department of Pathology and poultry diseases, College of Veterinary Medicine, University of Diyala, Iraq.

[2]Department of Public Health, College of Veterinary Medicine, University of Diyala, Iraq.

[3]Department of Internal medicine, College of Veterinary Medicine, University of Diyala, Iraq.

*Corresponding author: anashumady@yahoo.com

ABSTRACT

Several previous studies have been directed to find out the antioxidant effects of resveratrol against Cadmium chloride in many laboratories animals like rats and mice, the rabbits (12 rabbits) were separated equally and randomly into 3 groups each group consist of 4 rabbits, the first group (control group) received a normal rabbit pellets, the 2nd group (Cd group) received orally by stomach tube 30 mg / kg of Cadmium twice weekly for 60 days, while 3rd group (group Cd+ RES) administration orally by stomach tube 30 mg / kg of Cadmium twice weekly for 60 days, and after 1 hour administration orally by stomach tube 500 mg/ kg of resveratrol twice weekly for 60 days, the outcomes showed significant increase of Thiobarbituric acid reactive substances (TBARS) in 2nd group while the superoxide dismutase & glutathione peroxidase showed significant decrease in 2nd group, the results of pathological changes on lung in 2nd group showed fibrosis, disruption of alveolar wall, emphysema, infiltration of inflammatory cells and collapse, not important lesions in 3rd group.

Keywords: Resveratrol, Antioxidant, cadmium chloride, Lung tissue.

INTRODUCTION

Cadmium (Cd) is an inorganic toxicant of significant environmental and working concern. The general populace may be unprotected to cadmium via contaminants found in food and drinking water, inhaling particulates from ambient air or tobacco smoke, or ingesting contaminated soil or dust (Waalkes, 2000; Arriazu *et al.,* 2005; Arriazu *et al.,* 2006).

Cd is acknowledged to have a half-life of more than 20 years in humans. Cigarette smoke, and other sources like polluted water, air and food, are the primary causes of human intoxication. The respiratory and digestive systems are the most common targets for exposure. The liver, kidneys, respiratory system, reproductive system, and skeletal system are the key organs that are affected, reliant on the doses and exposure time (Jarup *et al.,* 1998).Inhalation of Cd dusts or exposure to Cd-contaminated air can cause acute injuries such as edema as well as chronic ailments including emphysema, pulmonary fibrosis, and adenocarcinomas (Amara *et al.,* 2011).

Red violet, berries, and peanuts are best sources of resveratrol (RES), a plant compound which serves as an antioxidant. This compound mainly concentrates on the skins and seeds of grapes and berries; RES has antioxidant, anti-inflammatory anti-platelet, anti-hyperlipidemic and anticancer properties. Therefore RES may play a role in preventing cancer, cardiovascular and

autoimmune diseases (Andrade & Ramalho, 2018; Colica et al., 2018; Salehi *et al.,* 2018; Xiao et al., 2019; Chen *et al.,* 2020; Meng *et al.,* 2020).

The commercial application of RES as a pharmaceutical medicine is now limited by a number of factors, the most significant of which are its poor bioavailability and quick metabolism. The poor solubility and bioavailability of RES appear to impact its in vivo actions in this regard. Over 70% of RES is absorbed by the gastrointestinal system after intake, however it is processed by three different metabolic routes, resulting in very poor bioavailability. The rate-limiting aspect in causal the bioavailability of RES appears to be extremely fast sulfate conjugation of RES in the intestine/liver (Walle *et al.,* 2004).

MATERIAL & METHODS

In this experiment,12 albino male rabbits, the age between 9-12 weeks and the weight between 450-650 gm,were taken from a Veterinarian's animal house. In The Vet. Medicine College of Diyala University, the animals were kept in plastic cages in an air-inured room; these plastic cages contained hardwood chips as bedding and the bedding was altered always to confirm a clean environment, rabbits were given food pellets and water ad libitum.

Chemicals: Cadmium chloride was purchased from Merck com. (Germany), The RES, was bought from Sigma Com (Louis, MO, USA).

Design of experiment: These rabbits were placed in three groups at random. Each group consisted of 4 rabbits, the 1st group (control group) received a normal rabbit pellet, the 2nd group (Cd group) received orally by stomach tube 30 mg/kg of Cadmium twice weekly for 60 days (ATSDR, 2008), while 3rd group (group Cd + RES) administration orally by stomach tube 30 mg/kg of Cadmium twice weekly for 60 days, and after 1-hour administration orally by stomach tube 500 mg/ kg of Resveratrol twice weekly for 60 days (Johnson *et al.,* 2011)

Blood collection: Blood was obtained from the heart using cardiac puncture procedure and placed in a test tube, which was then left for fiftten minutes to stance and coagulate in the refrigerator before being centrifuged at 500 rpm for 16 minutes to separate the serum and maintained at -20 C0 for evaluation.

Biochemical analysis: The biochemical parameters include the following

1- Thiobarbituric acid reactive substances (TBARS) (m moles/ dl) (Fraga *et al.,* 1998).

2- Superoxide dismutase (SOD) (U/ mg protein) (Kakkar *et al.,* 1984).

3- Glutathione peroxidase (GPx) (µg/ min/ mg protein) (Rotruck *et al.,* 1973).

Pathological examination: All animals was sacrifice under ethics protocol and slight anesthesia with ether, carefully dissect the lung and immediately fix it in 10% neutral formalin stored upwardly graded ethanol (50,75, 85, 90 and 100) first round , 2nd run, then xylene after that embedded in mold paraffin and stained with repetitive stains (hematoxylin and eosin (H&E) (Suvarna *et al.,* 2018).

Statistical analysis: The SPSS Version 17 software (2010) was used to statistically analyze all of the data. "Testing methods include one way ANOVA for comparisons among groups. P values of less than <0.05 were considered statistical significance. All data were expressed as means ± standard error (SE)" (Leech *et al.,* 2011).

RESULTS

Biochemical analysis:

The TBARS (m moles/ dl) showed a significant increase in the secend group linked with the 1st & 3rd group.

While the SOD displayed a significant reduction in the 2nd group compare with the 1st & 3rd group, it also showed significant differences between the 1st & 3rd group.

The GPx also presented a significant decrease in the 2nd group compared with the 1st & 3rd group and showed significant differences between the 1st & 3rd group.

Table (1): The level of TBARS in albino male rabbits.

groups	TBARS (m moles/ dl)
1st group (control group)	0.77 ± 0.10 b
2nd group (Cd) group	3.25 ± 0.24 a
3rd group (Cd + RES) group	0.55 ± 0.12 b

Number:4 for each group with considerably different (P<0.05).

Table (2): The level of SOD in albino male rabbits.

groups	SOD (U/ mg protein)
1st group (control group)	3.40 ± 0.19 a
2nd group (Cd) group	1.85 ± 0.20 c
3rd group (Cd + RES) group	2.35 ± 0.11 b

Number:4 for each group with considerably different (P<0.05).

Table (3): The level of GPx in albino male rabbits.

groups	GPx (µg/ min/ mg protein)
1st group (control group)	6.88 ± 0.25 a
2nd group (Cd) group	3.12 ± 0.30 c
3rd group (Cd + RES) group	5.15 ± 0.18 b

Number:4 for each group with considerably different (P<0.05).

Pathological changes: In the 3rd group, not significant any pathological changes, in 2nd group, exhibited line of fibrosis and slightly thickening of smooth musculature with

multiple emphysema in alveoli (fig. 1), also appear severe fibrosis, emphysema in alveoli, disruption of alveolar tissue with infiltration of inflammatory cells (fig. 2 & 3), thickening of interstitial layer, increased cellularity, infiltration of inflammatory cells with fibrosis (fig. 4), in other section showed emphysematous lung considered by diffuse dilation of the alveoli and interstitial thickening, hyperemia of the alveolar wall with congested BV., and infiltration of inflammatory cells (fig. 5 & 6), also showed severe fibrosis, the collapse of alveoli septa and infiltration of inflammatory cells (fig. 7), and finally appear degeneration of alveoli, present macrophage in interstitial with alveoli fibrous tissue and collapse of alveoli septa (fig. 8).

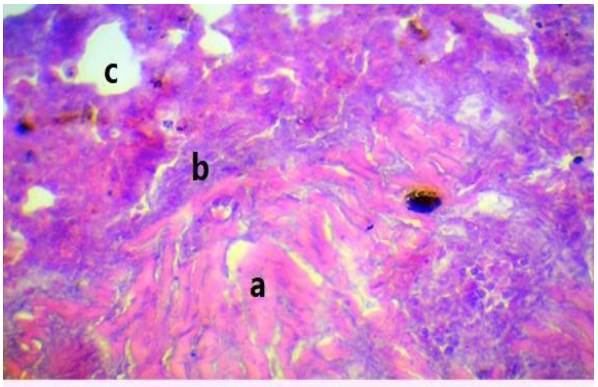

Figure (1): "Histopathological section" in the lung of albino male rabbits in 2nd group at day 60 revealed (a) fibrosis(b) condensing of smooth musculature (c) emphysemas lung (X40; H&E stain)

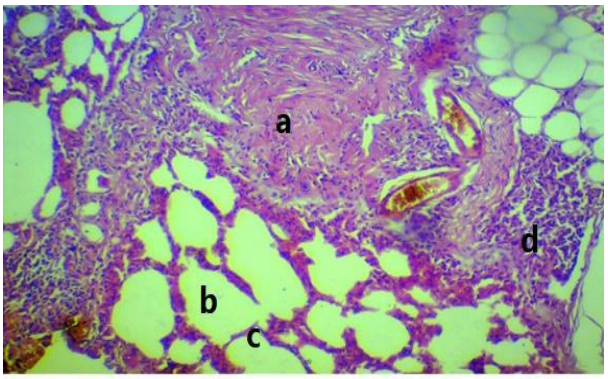

Figure (2): "Histopathological section" in the lung of albino male rabbits in 2nd group on day 60 revealed (a) severe fibrosis(b) emphysema in alveoli c) disruption of alveolar tissue d) penetration of inflammatory cells. (X40; H&E stain)

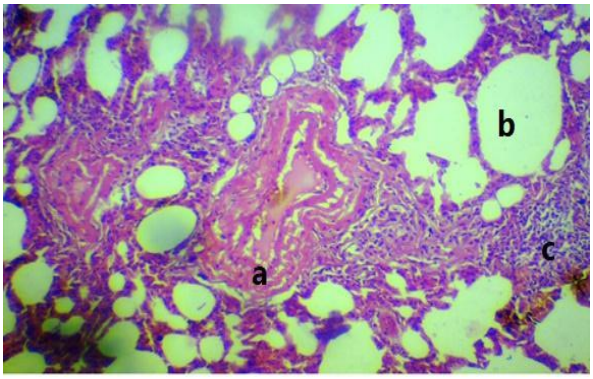

Figure (3): "Histopathological section" of the lung of albino male rabbits in 2nd group on day 60 showed a) fibrosis b) emphysema in alveoli c) inflammatory cells.
(X40; H&E stain)

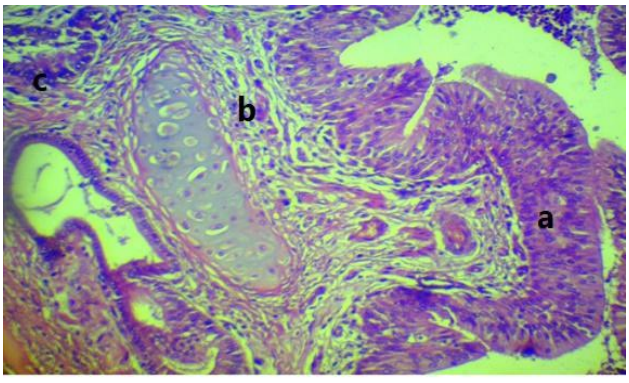

Figure (4): "Histopathological section" in the lung of albino male rabbits in 2nd group

on day 60 revealed (a) thickening of interstitial layer (b) increased cellularity (c) inflammatory cells. (X40; H&E stain)

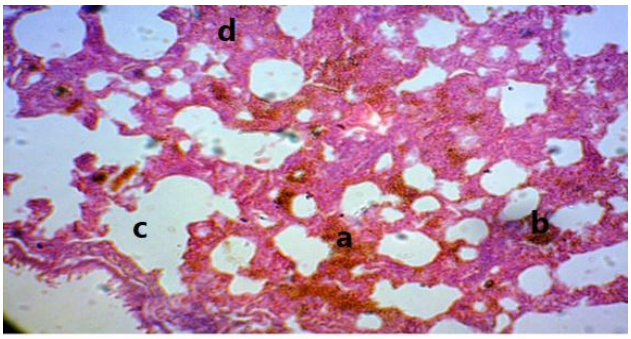

Figure (5): "Histopathological section" of the lung of albino male rabbits in 2nd group at day 60 revealed (a) hyperemia of alveoli(b) congested of BV (c) emphysema (d) stiffening in interstitial layer.

(X40; H&E stain)

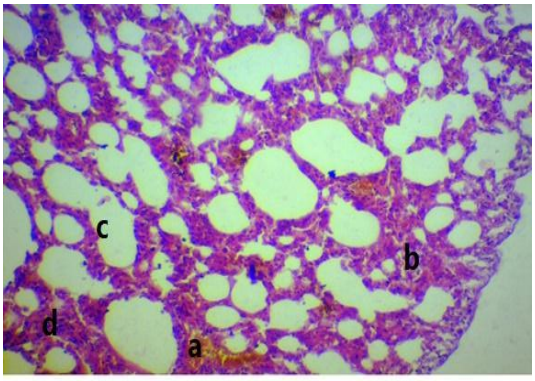

Figure (6): "Histopathological section" in the lung of albino male rabbits in 2nd group at day 60 revealed (a) hyperemia of lung alveolar wall (b) congestion of BV c) emphysema d) infiltration of inflammatory cells. (X40; H&E stain)

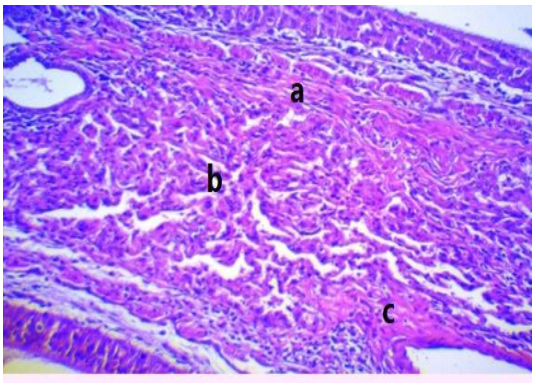

Figure (7): "Histopathological section" in the lung of albino male rabbits in 2nd group on day 60 revealed(a) severe fibrosis b) collapse of alveolar septa c) inflammatory cells.

(X40; H&E stain)

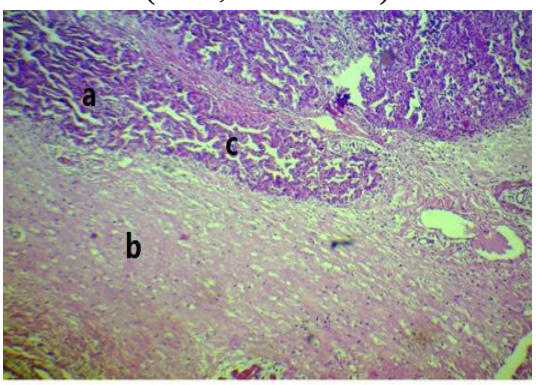

Figure (8): "Histopathological section" in the lung of albino male rabbits in 2nd group on day 60 showed a) degeneration of alveoli b) fibrosis c) collapse of alveoli septa

(X20; H&E stain)

DISCUSSION

The TBARS showed an increase in 2nd group treated by cadmium chloride that revealed a rise in lipid peroxidation levels, this result agrees with (Halliwell & Gutteridge, 1985; Cheng et al., 2006), who claims to have demonstrated that the Fenton transition equation accelerates the development of reactive oxygen species (ROS) and enhances the following iron and

copper-induced generation of lipid peroxidation and the highly reactive hydroxyl radical, The decrease in the activities of antioxidant enzymes (SOD, GPx) treated by cadmium chloride may be due to the inhibition of these enzymes by H2O and nitric oxide (NO) (Gstraunthaler *et al.,* 1983; Alam *et al.,* 2005).

Heavy metals in living beings can have a variety of negative consequences on the lungs, liver, brain, kidneys, testes, gastric, and intestinal systems (Nath *et al.,* 1984); the damage and pathological changes in 2nd group due to Cadmium can inactivate enzymes containing sulphydryl groups which lead to uncoupling of "oxidative phosphorylation in mitochondria & Cadmium" may also competes with calcium for the binding site on regulatory proteins or increase the metallothionein gene's transcription rate (Korotkov *et al*., 1996; Weaver *et al.,* 2004; Fekete & Brown, 2007). Also, the results in the 2nd group are on agreement with Humadi (2019), who demonstrated alterations in histopathology in lung, including hypertrophy of bronchial lymphoid tissu, edema, interstitial pneumonia, and shrinkage of alveoli septa after being treated by acrylonitrile. Also, the results are similar to those reported by (Owoeye *et al*., 2012), who detected variation in histopathology of lung of rats unprotected to dichlorvos.

Furthermore, the damage cells infiltrating were also mainly MNCs, on lung of 2nd group, these alterations may be result from the toxic effect of Cadmium chloride and phagocytosis by macrophages, dendritic and B lymphocytes that due to increasing the exposure to toxicity and infection (Al-Nailey, 2014; Humadi, 2019).

No significant pathological changes were seen in the third group due to the ability of resveratrol (RES) to act as an anti-inflammatory agent with a balanced control to increase total antioxidant status, defend from oxidative damage, prevent platelet aggregation, and improve lipid profile (Magyar et al., 2012; Colica et al., 2018). Moreover, the RES considers & performs anti-inflammatory activity through controlling & programming the action of transcription factor NF-kB in inflammatory reactions and the increase of cancer cells (Salehi *et al.,* 2018).

CONCLUSION

The resveratrol possesses significant antioxidant activity against the Cadmium chloride due to the presence of antioxidants and free radical scavenging activity, which prevent the production of cytokine storms.

REFERENCES:

1- Arriazu R, Pozuelo JM, Henriques-Gil N, Perucho T, Martin R, Rodriguez R and Santamaria L (2006). Immunohistochemical study of cell proliferation, Bcl-2, p53, and caspase- 3 expression on preneoplastic changes induced by cadmium and zinc chloride in the ventral rat prostate. J Histochem. Cytochem. 981-990.

2- Arriazu R, Pozuelo JM, Martin R, Rodriguez R and Santamaria L (2005). Quantitative and immunohistochemical evaluation of PCNA, androgen receptors, apoptosis, and Glutathione- S-Transferase P1 on preneoplastic changes induced by

cadmium and zinc chloride in the rat ventral prostate. Prostate. 347-357.

3- Waalkes MP (2000). Cadmium carcinogenesis in review. J Inorg. Biochem. 241-244.

4- Colica, C.; Milanovi´c, M.; Mili´c, N.; Aiello, V.; De Lorenzo, A.; Abenavoli, L. (2018). A Systematic Review on Natural Antioxidant Properties of Resveratrol. Nat. Prod. Commun. 13.

5- Andrade, S.; Ramalho, M.J.; Pereira, M.D.C.; Loureiro, J.A. (2018). Resveratrol Brain Delivery for Neurological Disorders Prevention and Treatment. Front. Pharm. 9: 1261.

6- Xiao, Q.; Zhu,W.; Feng,W.; Lee, S.S.; Leung, A.W.; Shen, J.; Gao, L.; Xu, C. (2019). A Review of Resveratrol as a Potent Chemoprotective and Synergistic Agent in Cancer Chemotherapy. Front. Pharmacol. 9.

7- Walle, T.; Hsieh, F.; DeLegge, M.H.; Oatis, J.E., Jr.;Walle, U.K. (2004). High absorption but very low bioavailability of oral resveratrol in humans. Drug Metab. Dispos. 32: 1377–82.

8- Fraga CG, Leibovity BF, Topeel AL. (1998). *Free Rad Biol*, 4:155-61.

9- Kakkar P, Das B, Viswanathan PN. (1984). *Ind J Biochem Biophys*, 21:130-32.

10- Rotruck JT, Pope AL, Ganther HE, Swason AB, (1973). *Science*, 178:588-90.

11- Halliwell B, Gutteridge JMC, (1985). *Clarendon Press*, Oxford, UK.

12- Cheng JP, Hu WX, Liu XJ, Zeng M, Shi W, Wang WH, (2006). *J Environm Science*, 18:788-92.

13- Alam MM, Javed K, Jafir MA, J. (2005). Ethnopharmacol, 96:121-25.

14- Gstraunthaler G, Pfaller W, Kotanko P, (1983). Biochem Pharmacol, 31:2969-72.

15- Agency for Toxic Substances and Disease Registry (ATSDR) (2008). Toxicological Profile for Cadmium. Draft for public comment. US Department of Health and Human Services. Atlanta, US.

16- Amara S, Douki T, Garrel C, Favier A, Rhouma KB, Sakly M, Abdelmelek H (2011). Effects of static magnetic field and cadmium on oxidative stress and DNA damage in rat cortex brain and hippocampus. Toxicol. Ind. Health 27(2):99-106.

17- B. Salehi, A.P. Mishra, M. Nigam, B. Sener, M. Kilic, M. Sharifi-Rad, P.V.T. Fokou, N. Martins, J. Sharifi-Rad. (2018). Resveratrol: a double-edged sword in health benefits, Biomedicines 6 (3):91.

18- Jarup, L.; Berglund, M.; Elinder, CG.; Nordberg, G. and Vahter, M. (1998). Health effects ofcadmium exposure: a review of the literature and a risk estimate. Scand J Work Environ Health. 24:1–51.

19- M. Chen, Q. Fu, X. Song, A. Muhammad, R. Jia, Y. Zou, L. Yin, L. Li, C. He, G. Ye, C. Lv, X. Liang, J. Huang, M. Cui, Z. Yin. (2020). Preparation of resveratrol dry suspension and its immunomodulatory and anti-inflammatory activity in mice, Pharm. Biol. 58 (1): 8–15.

20- Suvarna, KS.; Layton, C. and Bancroft, JD. (2018). Bancroft's theory and practice of histological techniques E-Book. Elsevier Health Sciences.

21- X. Meng, J. Zhou, C.N. Zhao, R.Y. Gan, H.B. Li. (2020). Health benefits and molecular mechanisms of resveratrol: a narrative review, Foods 9 (3): 340.

22- Halliwell, B., Gutteridge, J. M. C., & Blake, D. (1985). Metal ions and oxygen radical reactions in human inflammatory

joint disease. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 311(1152), 659-671.
23- Nath R, Prasad R, Palinal VK, Chopra RK.(1984). Molecular basis of cadmium toxicity. Prog. Food Nutr. Sci. 8: 109–63.
24- Korotkov SM, Glazunov V, Rozengart EV, Suvorov AA.(1996). The action of organic cadmium complexes of different degrees of hydrophobicity on rat liver mitochondria. Tsitologiia 8: 075–83.
25- Weaver J, Porasuphatana S, Tsai P, Cao G, Budzichowski TA, Roman LJ, (2004). The effect of divalent cations on neuronal nitric oxide synthase activity. Toxicol. Sci. 1:25–31.
26- Fekete SG, Brown DL.(2007). Veterinary aspects and perspectives of nutrigenomics: a critical review. Acta. Vet. Hung 5: 29–39.
27- B. Salehi,; A.P. Mishra,; M. Nigam,; B. Sener, M. Kilic, M. Sharifi-Rad, P.V.T. Fokou, N. Martins, J. Sharifi-Rad, (2018). Resveratrol: a double-edged sword in health benefits, Biomedicines 6 (3): 91.
28- Magyar, K.; Halmosi, R.; Palfi, A.; Feher, G.; Czopf, L.; Fulop, A.; Battyany, I.; Sumegi, B.; Toth, K.; Szabados, E.(2012). Cardioprotection by resveratrol: A human clinical trial in patients with stable coronary artery disease. Clin. Hemorheol. Microcirc. 50:179–87.
29- Colica, C.; Milanovi´c, M.; Mili´c, N.; Aiello, V.; De Lorenzo, A.; Abenavoli, L.(2018). A Systematic Review on Natural Antioxidant Properties of Resveratrol. Nat. Prod. Commun.
30- Leech, N. L.; Barrett, K. C.; and Morgan GA.(2011). IBM SPSS For Intermediate statistics.4th ed.Taylor and Francis Group. LLC.USA.