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Therapeutic effect of rocket seeds (*Eruca sativa* L.) against hydroxyapatite nanoparticles injection induced cardiac toxicity in rats

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Abstract: Hydroxyapatite is bio-ceramic materials with a calcium to phosphorus proportion like to that of normal bone and teeth. The current study meant to examine the cardiac toxicity by hydroxyapatite nanoparticles (HAP NPs) and the protective effect of the rocket seeds in treatments. An aggregate of 40 male Wistar rodents were partitioned into 4 equal groups [Gp1, control; Gp2, rocket seeds extract (RS); Gp3, HAP NPs; Gp4, HAP NPs+RS]. Current results exhibit that; HAP NPs induce a significant increase in myoglobin, LDH (lactate dehydrogenase), CK-MB (creatinine kinase) and CK (creatinine kinase) levels, cardiac thiobarbituric acid-reactive substances (TBARS), injury and P53 expressions. In contrast; a significant reduction in cardiac catalase, reduced glutathione (GSH) and superoxide dismutase (SOD) as compared to control group. Post treatment of rat with HAP NPs and rocket seeds extract (HAP NPs+RS) improved the cardiac functions and structure. Rocket seeds extract may offer advantages against the harmful effects of hydroxyapatite nanoparticles.

Keywords: hydroxyapatite nanoparticles (HAP NPs); Rocket seeds extract; Heart; Oxidative stress; Injury and P53 expressions

INTRODUCTION

Hydroxyapatite is a bio-ceramic material with a calcium to phosphorus proportion as in normal teeth and bone. Therefore, great clinical interest in its utilization for bone grafts substitutes and coatings over metallic implants for bone recovery (Nayar *et al*., 2006). Hydroxyapatite can be synthesized using hydrolysis, precipitation, hydrothermal synthesis or mined from ordinary resources as bones of bovine and fishes, *crustacea* and shells or eggs (Heidari *et al*., 2015; Padmanabhan *et al*., 2015; Maleki-Ghale *et al*., 2016; Pal *et al*., 2017).

Nanoparticles (NPs) are particles that exist on a nanometre scale (i.e., beneath 100 nm in at any rate one measurement). They can have physical properties, for example, consistency, conductance or exceptional optical properties that make them alluring in materials science and science (Wang *et al*., 2011). Synthetic hydroxyapatite nanoparticles (HAP NPs) are progressively being utilized in clinical applications as a bioresorbable bearer material for controlled medication conveyance in the treatment of infections, osteoporosis and osteomyelitis (Hafez *et al*., 2012; Khajuria *et al*., 2015).

Investigations have revealed that hydroxyapatite nanoparticles (HAP NPs) powders possess improved densification and sinter ability due to increased surface area, which could ameliorate fracture toughness, and other mechanical properties (Abd El-Fattah *et al*., 2014). Moreover, HAP NPs, contrasted with HAP, indicated positive impact on cell expansion of human osteoblast-like cells *in vitro* and animated hard tissue recovery in vivo (Zhou and Lee, 2011). All things considered, the specific role of HAP NPs in bone repair, especially the real correlation between HAP NPs and main cells responsible for bone regeneration, is a long way from being explained and the biocompatibility of HAP NPs is as yet a matter of discussion (Shi *et al*., 2009; Zhu *et al*., 2013).

Recently, there is a marked increase in the utilization of correlative and elective medication (El-Moghazy *et al*., 2014; Moustafa *et al*., 2014; Tousson *et al*., 2018a, 2018b, 2020; El Masry *et al*., 2018, 2019, 2020; Abd Eldaim *et al*., 2019). Numerous plants items have huge cancer prevention agent exercises, which assume significant jobs in numerous diseases treatment (Mutar *et al*., 2019; Eldaim *et al*., 2019; Aldubayan *et al*., 2019). The rocket plant (*Eruca sativa* L.) is a herbaceous yearly vegetable for the most part known as the "plate of mixed greens herb" has a place with the group of Brassicaceae and is local to the Mediterranean Region and West Asia (Vieira *et al*., 2015). Plant-determined cancer prevention agents have free-radical rummaging exercises and are profoundly created in blossoms and leaves in light of oxidative

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pressure and senescence degenerative procedures (Cavaiuolo *et al*., 2013). Among all the components, Mg, Ca, Fe and K are the most bottomless minerals in the rocket leaves; however Na, Ca, Cr and P are pervasive in the rocket seeds (Bukhashi *et al*., 2007).

Nanoparticles are known to actuate receptive oxygen species (ROS) creation, prompting an oxidative pressure when redox condition of the cell is imbalanced (Sarkar *et al*., 2014). ROS acceptance by nanoparticles is viewed as the essential driver of nanotoxicity, and has been ascribed to the nearness of star oxidant practical gatherings on their responsive surface or due to nanoparticle-cell communications (Risom *et al*., 2005). The current study planned to explore the therapeutic role of rocket seeds (RS) against the injection of hydroxyapatite nanoparticles (HAP NPs) in rat induced cardiac damage by decreasing oxidative stress and apoptosis.

MATERIALS AND METHODS

Hydroxyapatite nanoparticles

Hydroxyapatite nanoparticles (HAP NPs) and the dose of hydroxyapatite nanoparticles was 300mg/kg BW (dissolved in distilled water)) >30 nm particle size were bought from nano-tech Company (Nanotech Egypt).

Animals and experimental design

40 Wistar male rats weighing 160-170g were involved in this study. Animals were obtained from Faculty of Medicine, Alexandria University, Egypt. Throughout the treatment time (8 weeks), rats were divided to four groups [Gp1, control; Gp2, treated rats with RS (intragastrically, 30 mg/kg body weight/day); Gp3, HAP NPs (IP; 300 mg/kg BW /day) for four weeks; Gp4, HAP NPs+RS (HAP NPs for four weeks and then treated with RS for another four weeks)]. Rata conservation and treatments were conducted in accordance with the Faculty of Science, Tanta University guide for animal, as approved by Institutional Animal Care and Use Committee (IACUC – SCI - TU - 0129).

Tissue preparation

Rats from each gathering were fasting, euthanized and exposed to a total necropsy. Heart tissues were weighed, cut and homogenized (10% w/v) according to Salama *et al*. (2014).

Cardiac biomarkers

Serum LDH and myoglobin levels were detected according to Tousson *et al*. (2018b) and Muller-Bardorff *et al*. (2000) respectively. Serum CK-MB and CK levels were detected after Bishop *et al*. (1971) and Zilva and Pannall (1988) respectively.

Assessment of cardiac oxidative stress biomarkers

The strategy suggested by Oyouni *et al*. (2018) was applied for determination of TBARS within the cardiac homogenate. Ibrahim *et al*. (2011) were used to determine the concentrations of catalase enzyme activity, the methodology reported by Saggu *et al*. (2014) was used to determine levels of superoxide dismutase (SOD) within the cardiac homogenate and the approach by Oyouni *et al*. (2018) helped with the measurement of reduced glutathione (GSH).

Histopathological Examination

Prepared for paraffin sectioning and subjected to histopathological examination using haematoxylin and eosin stains according to Tousson (2016) and Tousson *et al*. (2012).

Immunohistochemical detection of P53

P53 immunoreactivities were detected in heart sections using ABC method after Tousson *et al*. (2014).

Ethical approval

Animal maintenance and treatments were conducted in accordance with the Faculty of Science, Tanta University guide for animal, as approved by Institutional Animal Care and Use Committee (IACUC -SCI - TU - 0129

STATISTICAL ANALYSIS

Data were reported as mean ± SE and one way ANOVA was used to detect significant ($p<0.01$) differences between treatment groups. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® Inc., USA).

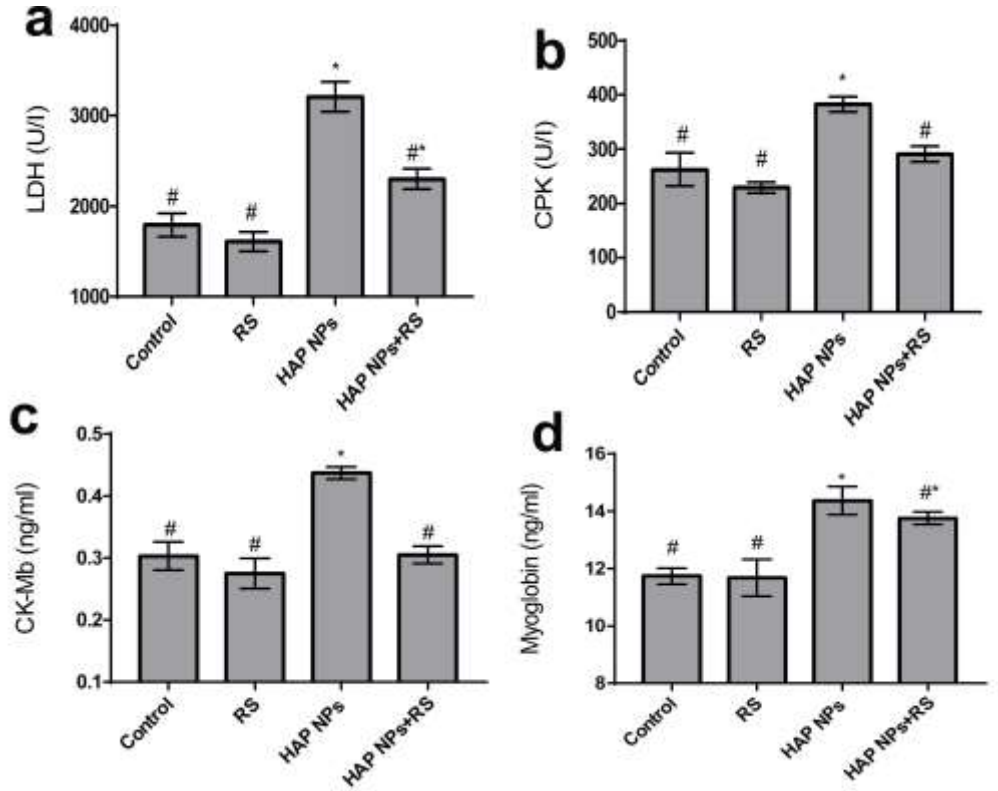
RESULTS

Changes in cardiac functions in different groups under study

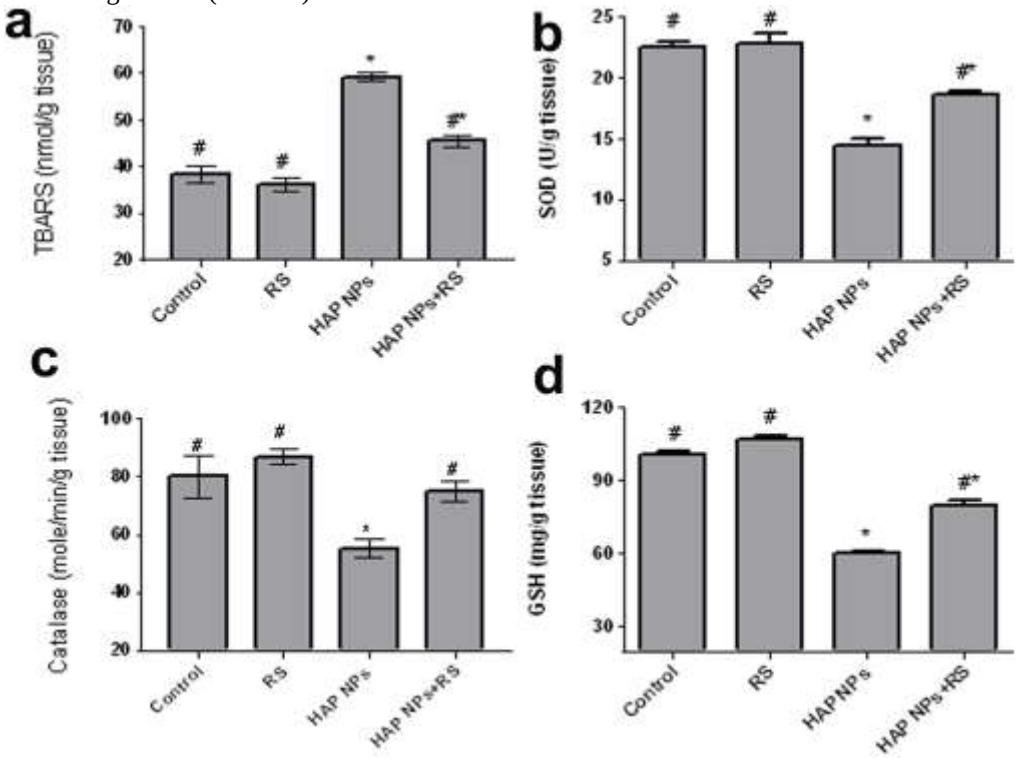
Compared to the control and RS groups, HAP NPs group showed a significant elevation in cardiac markers (CPK, CK-Mb, LDH and myoglobin), but using both HAP NPs and rocket seeds extract (HAP NPs+RS) significantly depletion those levels (figs. 1a-1d).

Oxidative and antioxidant enzyme activities

Fig. 2 revealed that; a significant elevation in TBARS and significant decrease in GSH in cardiac tissues of HAP NPs group relative to control and rocket seeds. Furthermore, rats treated with rocket seeds extract after HAP NPs exhibits significant depletion in TBARS and significant elevation in GSH as compared with HAP NPs group (figs. 2a & 2d). Fig. 2 (b&c) revealed that; a significant depletion in cardiac SOD and CAT activities in HAP NPs group when compared with control and rocket seeds groups. Furthermore, rats treated with rocket seeds extract after HAP NPs exhibits a significant elevation in cardiac SOD and CAT activities as compared with HAP NPs group (figs. 2 & 2c).



Figs. 1(a-d): Variations in the cardiac function markers (CPK, CK-Mb, LDH and myoglobin) in the experimental groups; expression of values were expressed as means ± SE, with 10 subjects in every treatment group; # and * respectively denote significant (P< 0.01) difference from HAP NPs and from control.



Figs. 2(a-d): Variations in the oxidative stress parameters (TBARS, catalase, GSH, and SOD) in heart tissues in experimental groups; expression of values were expressed as means ± SE with 10 subjects in every treatment group; # and * respectively denote significant (P< 0.01) difference from HAP NPs and from control.

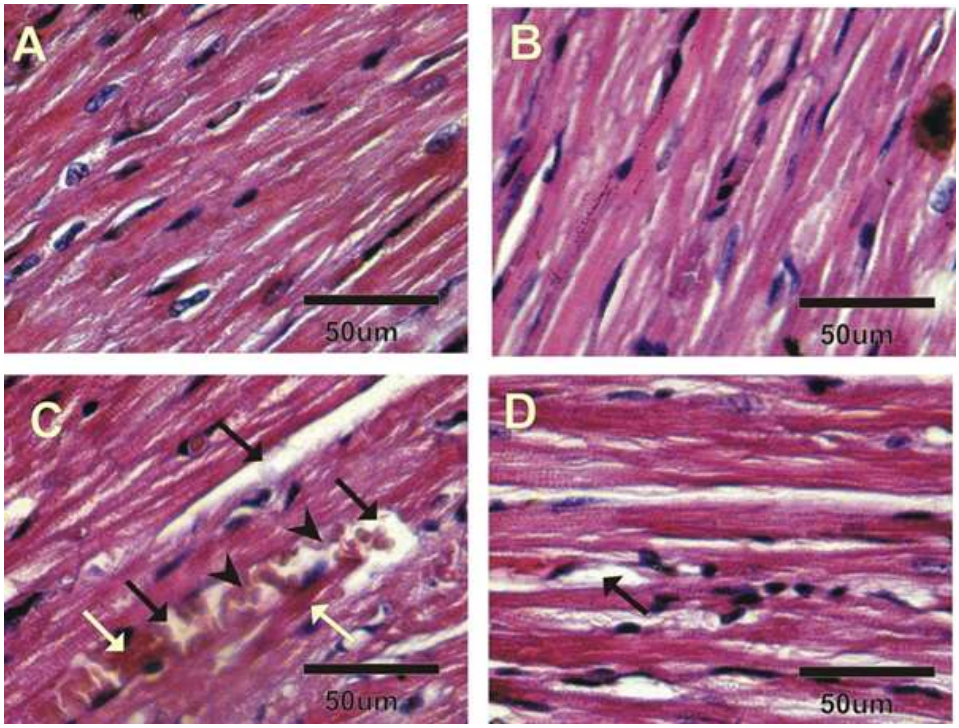


Fig. 3: Heart sections photomicrographs stained with H&E. A) and B) Heart sections in control and treated rats with rocket seeds extract groups showed normal myofibrillar structure with striations. C) Heart sections in treated rats with HAP NPs revealed moderate to marked myocardial hypertrophy (Black arrows), cytoplasmic vacuoles, and focal hemorrhage (White arrows). D) Heart sections in post treated rats with HAP NPs and rocket seeds root extract revealed mild cytoplasmic vacuoles and mild myocardial hypertrophy (Black arrows).

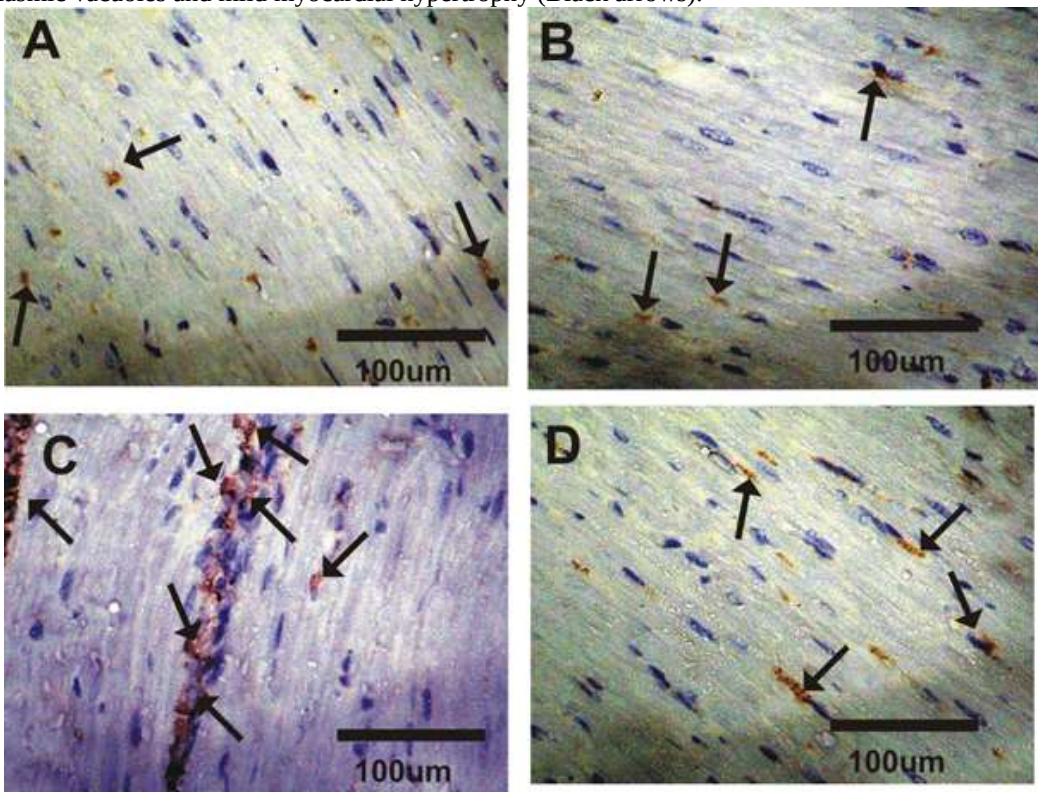


Fig. 4: Photomicrographs of heart sections stained with apoptotic P53 markers. A) and B) Mild positive reactions for P53 (arrows) in the control and rocket seeds groups. C) Moderate to marked positive reactions for P53 in HAP NPs group. D) Moderate to mild positive reactions for P53 in heart sections in HAP NPs+RS group (arrows).

Heart histopathology

Heart sections in control and rocket seeds groups showed normal myofibrillar structure with striations (figs. 3A & 3B). In contrast; heart sections in HAP NPs group revealed moderate to marked hypertrophy, vacuolations, focal hemorrhage, and nuclear pyknosis (fig. 3C). Heart sections in HAP NPs + RS group exhibit myocardial improvement with only mild injury, and mild hypertrophy (fig. 3D).

P53 immunoreactivity

Heart sections in control and rocket seeds groups exhibits mild positive reactions for P53 (figs. 4A&4B) while; moderate to marked positive reactions for P53 were observed in HAP NPs group (fig. 4C). Furthermore; moderate to mild positive reactions for P53 were shows in heart sections in HAP NPs + RS group (fig. 4D).

DISCUSSION

Nanoparticles induced respiratory, cardiovascular, lymphatic, autoimmune, neurodegenerative diseases and a variety of cancers that can manifest immediately following exposure or many years later. Relatively few studies have been done to examine the effect of nanoparticles on the cardiovascular system. Recently, several studies have been done to examine the effect of natural antioxidant plants on cardiovascular dieses (Salama *et al*. 2013, 2015; Tousson *et al*., 2014, 2020; Abd Eldaim *et al*., 2019). So, the current study was planned to examine the rocket seeds extract role in improving rat cardiac toxicity by hydroxyapatite nanoparticles.

Current results revealed that; treated rats with HAP NPs induced significant elevation in the cardiac markers (CK, CK-Mb, LDH and myoglobin) levels by comparison to the control and RS groups, in contrast; a significant depletion in cardiac markers after treatment (HAP NPs+RS) as compared to HAP NPs group. Our results adhere with Duan *et al*. (2009) who demonstrated that; TiO2 NPs increase in CK, AST, LDH, and HBDH levels indicators of myocardial injury. Also, Bu *et al*. (2010) showed that TiO2 NPs-treated rats elevated AST, CK, LDH, troponin T, myoglobin, and CK-MB. Also our results are in alignment with Bostan *et al*. (2016) who informed that; administration of silica NPs to rats induced elevation in CRP, CK-MB, LDH, and D-dimer that cause inflammation in cardiovascular system. These data are supported by Chen *et al*., (2006) who reported that; copper nanoparticles has hepatic toxicity in adult mice. Also; Xiong *et al*. (2015) who reported that; iron oxide nanoparticles (IONPs) treated rats induced an increased in LDH, CK, CK-MB and MDA activities and decrease SOD activity.

Current results exhibit elevation in TBARS and depletion in catalase, GSH and SOD activities in HAP NPs group. Further more; these activities were improved in HAP NPs+RS group. The present study is in covenant with Turkez *et al*. (2014) who find that; the exposure to HAP NPs induce oxidative stress, with an elevation of the H2O2 and MDA levels and a depletion in the SOD and GSH. Also with Xu *et al*. (2012) who found that hydroxyapatite nanoparticles induced intracellular accumulation of ROS and decreased SOD in C6 cells. Turkez *et al*. (2014) reported that; exposure to HAP NPs led to increases of total oxidative stress and decrease total antioxidant capacity in blood cells culture in human.

CONCLUSIONS

Hydroxyapatite nanoparticles induced cardiac toxicity, tissue injury, oxidative stress and apoptosis, alterations. rocket seeds extract may offer advantages against the dangerous nature of hydroxyapatite nanoparticles.

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REFERENCES

Abd Eldaim M, Tousson E, El Sayed I and Awd WM (2019). Ameliorative effects of saussurea lappa root aqueous extract against ethephon - induced reproductive toxicity in male rats. *Environm. Toxicol*., **34**(2): 150-159.

Abd El-Fattah HE, Helmy YB, El-Kholy B and Marie M (2010). In vivo animal histomorphometric study for evaluating biocompatibility and osteointegration of nano-hydroxyapatite as biomaterials in tissue engineering. *J. Egypt. Nat. Cancer Inst*., **22**(4): 241-50.

Aldubayan MA, Elgharabawy RM, Ahmed AS and Tousson E (2019). Antineoplastic Activity and Curative Role of Avenanthramides against the Growth of Ehrlich Solid Tumors in Mice. *Oxid. Med. Cellular Long*., **2019**. ID 5162687, https://doi.org/10.1155/2019/5162687

Bishop C, Chu TM and Shihabi ZK (1971). Single stable reagent for creatine kinase assay. *Clinical chem*., **17**(6): 548-550.

Bostan HB, Rezaee R, Valokala MG, Tsarouhas K, Golokhvast K, Tsatsakis AM and Karimi G (2016). Cardiotoxicity of nano-particles. *Life Sci.,* **165**(1): 91-99.

Bu Q, Yan G, Deng P, Peng F, Lin H, Xu Y, Cao Z, Zhou T, Xue A, Wang Y and Cen X (2010). NMR-based

metabonomic study of the sub-acute toxicity of titanium dioxide nanoparticles in rats after oral administration. *Nanotechnol*., **21**(12): 125105.

Bukhsh E, Malik SA and Ahmad SS (2007). Estimation of nutritional value and trace elements content of Carthamus oxyacantha, *Eruca sativa* and *Plantago ovata*. *Pak. J. Botany*, **39**(4):1181.

Cavaiuolo M, Cocetta G and Ferrante A (2013). The antioxidants changes in ornamental flowers during development and senescence. *Antioxidants*, **2**(3): 132-55.

Chen Z, Meng H, Xing GM, Chen CY, Zhao YL, Jia G, Wang TC, Yuan H, Ye C, Zhao F, Chai ZF, Zhu CF, Fang XH, Ma BC and Wan LJ (2006). Acute toxicological effects of copper nanoparticles in vivo. *Toxicol. Lett.*, **163**(2): 109-120

Duan Y, Liu H, Zhao J, Liu C, Li Z, Yan J, Ma L, Liu J, Xie Y, Ruan J and Hong F (2009). The effects of nano-anatase TiO 2 on the activation of lactate dehydrogenase from rat heart. *Biological Trace Element. Res.*, **130**(2): 162-71.

Eldaim M, Tousson E, El Sayed IE, El Ae and Elsharkawy HN (2019). Grape seeds proanthocyanidin extract ameliorates Ehrlich solid tumor induced renal tissue and DNA damage in mice. *Biomed. & Pharmacoth*., **115:** 108908

El-Masry T, Al-Shaalan N, Tousson E, Buabeid M and Al-Ghadeer A (2020). Potential therapy of vitamin B17 against Ehrlich solid tumor induced changes in Interferon gamma, Nuclear factor kappa B, DNA fragmentation, p53, Bcl2, survivin, VEGF and TNF-α Expressions in mice. *Pak. J. Pharm. Sci*., **33**(1): 393-401.

El-Masry TA, Al-Shaalan NH, Tousson E, Buabeid M and Alyousef AM (2019). The therapeutic and antineoplastic effects of vitamin B17 against the growth of solid-form Ehrlich tumours and the associated changes in oxidative stress, DNA damage, apoptosis and proliferation in mice. *Pak. J. Pharm. Sci.*, **32**(6): 2801-2810.

Elmasry TA, Al-Shaalan NH, Tousson E, El-Morshedy K and Al-Ghadeer A (2018). Star anise extracts modulation of reproductive parameters, fertility potential and DNA fragmentation induced by growth promoter equigan in rat testes. *Braz. J. Pharm. Sci.*, **54**(1): https://doi.org/10.1590/s2175-97902018000117261

El-Moghazy M, Zedan NS, El-Atrsh AM, El-Gogary M and Tousson E (2014). The possible effect of diets containing fish oil (omega-3) on hematological, biochemical and histopathogical alterations of rabbit liver and kidney. *Biomed. & Preventive Nutr*., **4**(3): 371-377.

Hafez AI, Hafez F, Khedr M, Ibrahim O, Sabry R and Abdel-Wahhab MA (2012). Safety evaluation of needle-like hydroxyapatite nanoparticles in female rats. *Egypt Pharmaceutical. J*., **11**(2): 67.

Heidari F, Bahrololoom ME, Vashaee D and Tayebi L (2015). *In situ* preparation of iron oxide nanoparticles in natural hydroxyapatite/chitosan matrix for bone tissue engineering application. *Ceramics Intern*., **41**(2): 3094-3100.

Khajuria DK, Razdan R and Mahapatra DR (2015). Development, *in vitro* and *in vivo* characterization of zoledronic acid functionalized hydroxyapatite nanoparticle based formulation for treatment of osteoporosis in animal model. *Eur. J. Pharmaceutical Sci*., **66**(1): 173-183.

Ibrahim W, Tousson E, Ali EM and Mansour MA (2011). Folic acid alleviates oxidative stress and hyperhomocysteinemia involved in testicular dysfunction of hypothyroid rats. *Gen. Comp. Endocrinol*., **174**(2): 143-9.

Maleki-Ghale H, Aghaie E, Nadernezhad A, Zargarzadeh M, Khakzad A, Shakeri MS, Beygi Khosrowshahi Y and Siadati MH (2016). Influence of Fe3O4 Nanoparticles in Hydroxyapatite Scaffolds on Proliferation of Primary Human Fibroblast Cells. *J. Mater Eng. Perform.*, **25**(6): 2331-2339.

Moustafa A, Ali E, Moselhey S, Tousson E and El-Said K (2014). Effect of coriander on thioacetamide-induced hepatotoxicity in rats. *Toxicol. Industrial Health*, **30**(7): 621-9.

Müller-Bardorff M, Sylven C, Rasmanis G, Jørgensen B, Collinson PO, Waldenhofer U, Hirschl MM, Laggner AN, Gerhardt W, Hafner G and Labaere I (2000). Evaluation of a point-of-care system for quantitative determination of troponin T and myoglobin. *Clinical chemistry and laboratory medicine.* **38**(6): 567-574.

Mutar TF, Tousson E, Hafez E, Gazia MA and Salem SB (2020). Ameliorative effects of vitamin B17 on the kidney against Ehrlich ascites carcinoma induced renal toxicity in mice. *Environm. Toxicol*., **35**(4): 528-537.

Nayar S, Sinha MK, Basu D and Sinha A (2006). Synthesis and sintering of biomimetic hydroxyapatite nanoparticles for biomedical applications. *J. Materials Sci. Materials in Med.*, **17**(11): 1063-1068.

Oyouni AA, Saggu S, Tousson E and Rehman H (2018). Immunosuppressant drug tacrolimus induced mitochondrial nephrotoxicity, modified PCNA and Bcl-2 expression attenuated by *Ocimum basilicum* L. in CD1 mice. *Toxicol. Report*., **5**(1): 687-694.

Risom L, Møller P and Loft S (2005). Oxidative stress-induced DNA damage by particulate air pollution. *Mutation Res./Fundamental Molec. Mechan. of Mutagen.*, **592**(1-2): 119-137.

Saggu S, Sakeran M, Zidan N, Tousson E, Mohan A and Rehman H (2014). Ameliorating effect of chicory (*Chichorium intybus* L.) Fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. *Food Chem. Toxicol*., **72**(1): 138-146.

Salama A, Tousson E, Ibrahim W and Hussein W (2013). Biochemical and histopathological studies of the ptu-induced hypothyroid rat kidney with reference to the

ameliorating role of folic acid. *Toxicol. Industrial Health*, **29**(7): 600-8.

Salama AF, Tousson E, Shalaby KA and Hussien HT (2014). Protective effect of curcumin on chloroform as by-product of water chlorination induced cardio-toxicity. *Biomed. & Prevent. Nutr*., **4**(2): 225-230.

Salama AF, Kasem SM, Tousson E and Elsisy MK. (2015). Protective role of L-carnitine and vitamin E on the testis of atherosclerotic rats. *Toxicol. Industrial Health*, **31**(5): 467-474.

Sarkar A, Ghosh M and Sil PC (2014). Nanotoxicity: Oxidative stress mediated toxicity of metal and metal oxide nanoparticles. *J. Nanosci. Nanotechnol*., **14**(1): 730-743.

Shi Z, Huang X, Cai Y, Tang R and Yang D (2009). Size effect of hydroxyapatite nanoparticles on proliferation and apoptosis of osteoblast-like cells. *Acta Biomaterialia*. **5**(1): 338-345.

Tousson E (2016). Histopathological alterations after a growth promoter boldenone injection in rabbits. *Toxicol. Industrial Health*, **32**(2): 299-305.

Tousson E, Ali EM, Ibrahim W and Ashraf RM (2012). Histopathological and immunohistochemical alterations in rat heart after thyroidectomy and the role of hemin and ketoconazole in treatment. *Biomed. & Pharmacoth*., **66**(8): 627-632.

Tousson E, Bayomy MF and Ahmed AA (2018a). Rosemary extract modulates fertility potential, DNA fragmentation, injury, KI67 and P53 alterations induced by etoposide in rat testes. *Biomed. & Pharmacoth*., **98**(1): 769-774.

Tousson E, Elgharabawy RM, Elmasry TA. (2018b) Grape seed proanthocyanidin ameliorates cardiac toxicity induced by boldenone undecylenate through inhibition of NADPH oxidase and reduction in the expression of NOX2 and NOX4. Oxidative Med Cellular Long. **2018**;2018. Article ID 9434385, 12 pages, https://doi.org/10.1155/2018/9434385

Tousson E, Hafez E, Gazia MM, Salem SB and Mutar TF (2020). Hepatic ameliorative role of vitamin B17 against Ehrlich ascites carcinoma–induced liver toxicity. *Environm. Sci. Pollution Res.*, **27**(1): 9236-9246

Tousson E, Hafez E, Zaki S and Gad A (2014). P53, Bcl-2 and CD68 expression in response to amethopterin-induced lung injury and ameliorating role of l-carnitine. *Biomed. & Pharmacoth*., **68**(5): 631-639.

Turkez H, Yousef MI, Sönmez E, Togar B, Bakan F, Sozio P and Stefano AD (2014). Evaluation of cytotoxic, oxidative stress and genotoxic responses of hydroxyapatite nanoparticles on human blood cells. *J. Appl. Toxicol*., **34**(4): 373-379.

Vieira JF, Oliveira SD, Zanatta ZC, Lemes ES, Villela FA and Barros AC (2015). Physiological and phytosanitary potential of rocket seeds. *Ciência Rural*. **45(**2):200-5.

Wang H, Wu J, Sun S, Liu B, Cheng F, Sun R and Wang X (2011). Glucosinolate biosynthetic genes in Brassica rapa. *Gene*. **487**(2): 135-142.

Xiong F, Wang H, Feng Y, Li Y, Hua X, Pang X, Zhang S, Song L, Zhang Y and Gu N (2015). Cardioprotective activity of iron oxide nanoparticles. *Scientific Reports*. **5**(1): 1-8.

Xu J, Xu P, Li Z, Huang J and Yang Z (2012). Oxidative stress and apoptosis induced by hydroxyapatite nanoparticles in C6 cells. *J. Biomed. Materials Res. Part A*. **100**(3): 738-745.

Zhou H and Lee J (2011). Nanoscale hydroxyapatite particles for bone tissue engineering. *Acta Biomaterialia*. **7**(7): 2769-2781.

Zhu W, Wang D, Peng L, Zhang X, Ou Y, Fen W, Lu W, Han Y and Zeng Y (2013). An experimental study on the application of radionuclide imaging in repairing bone defects. *Artificial Cells, Nanomed. Botechnol*., **41**(5): 304-308.

Zilva JF, Pannall PR and Mayne PD (1988). Clinical Chemistry in Diagnosis and Treatment. Year Book Medical Pub; 1988.