doi.org/10.36721/PJPS.2020.33.3.SUP.1251-1260.1

Chicory (*Cichorium intybus* L.) extract ameliorates hydroxyapatite nanoparticles induced kidney damage in rats

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Abstract: Hydroxyapatite nanoparticles (HAP NPs) are a calcium phosphate, basically and artificially like the mineral period of human bone and teeth. Current study were performed to study the therapeutic effects of chicory extract towards the injection of hydroxyapatite nanoparticles (HAP NPs) in rat induced kidney damage by decreasing kidney functions, electrolytes, cytokines and apoptosis. An aggregate of 40 Wistar male rats were divided into 4 equal groups [control, chicory, HAP NPs, treated HAP NPs with chicory (HAP NPs+chicory)]. Rat treated with HAP NPs exhibit increased serum creatinine, urea, cystatin C, potassium ions, chloride ions, kidney injury, Bax, PCNA and TNFα expression. In contrast; decline in serum sodium and calcium ions. Post treatments of HAP NPs with chicory extract (HAP NPs+chicory) improved the kidney structure and functions. We can conclude that; chicory extract may offer advantages against the dangerous nature of hydroxyapatite nanoparticles (HAP NPs).

Keywords: Hydroxyapatite nanoparticles (HAP NPs), Chicory extract, kidney, Bax, PCNA and TNFα expressions.

INTRODUCTION

Nanoparticles (NPs) commonly running in estimation from 1-100 nanometers (nm) have properties exceptional from their diminishing molecule and mass proportionate sizes of any component to the levels of atomic variations, it tends to be controlled to be utilized for some gainful requests (Dolez and Debia, 2015; Yousef *et al*., 2019). Engineered nanomaterials have discovered applications in numerous parts, including car, synthetics, development, beauty care products, vitality, building, condition, medication, security, sports, media transmission, materials and transportation (Dolez and Debia, 2015; Altwaijry *et al*., 2020).

Hydroxyapatite ((HAP); Ca10(PO4)6(OH)2) is a bio-ceramic materials with a calcium to phosphorus proportion as in normal teeth and bone (Nayar *et al*., 2006; Khajuria *et al*., 2015). Hydroxyapatite can be synthesized using hydrolysis, precipitation, hydrothermal synthesis or extracted from natural resources as fish bones, seashells, bovine bones, eggshells or shrimp shells (Heidari *et al*., 2015; Padmanabhan *et al*., 2015; Maleki-Ghale *et al*., 2016; Pal *et al*., 2017).

Hydroxyapatite nanoparticles (HAP NPs) are seen as an effective impetus for union of 2, 3 – dihydro-quinazolin-4 (1H) single subsidiaries in watery media by means of a 3 part one - pot buildup of isatoic anhydride and fragrant aldehydes with essential amines (Oberbek *et al*., 2018). HAP NPs is a calcium phosphate, basically and artificially like the mineral period of human bone and teeth. Because of its high biocompatibility and bioactivity, it has been effectively applied in the assembling of beautifying agents and cleanliness items, just as in bone-tissue designing and regenerative medication. The utilization of nanosized hydroxyapatites in biomedical applications is continually becoming because of their great mechanical properties and improved effectiveness of quality transfection in sedate conveyance (Abd El-Fattah *et al*., 2014; Simon *et al*., 2019).

Recently, there is a marked elevation in the utilization of corresponding and elective medication (El-Moghazy *et al*., 2014; Moustafa *et al*., 2014; Tousson *et al*., 2018, 2020; El Masry *et al*., 2018, 2019, 2020; Aldubayan *et al*., 2019; Mutar *et al*., 2019). Kidney diseases is accompanying with extraordinary morbidity and mortality, and these diseases are associated with elevation oxidative damage, and endogenous and synthetic antioxidants (Salama *et al*., 2012, 2013; El-Moghazy *et al*., 2014; Oyouni *et al*., 2018; Tousson *et al*., 2019; Eldaim *et al*., 2019).

Cichorium intybus is a perennial herb and medicinally important plant that belongs to the family Asteraceae that has been used in Arab country and many centuries in

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Europe (Rumball, 1986; Rumball *et al*., 2003). *Cichorium intybus*, generally identified as chicory is usually used as substitute for coffee. Also, it had been used commonly for treatment of many disorders from lesions to diabetes. This plant is used for treatment of many diseases like tachycardia, cancer, impotence, dysmenorrhoea, diabetes, insomnia, splenitis and AIDS (Duke, 1983; Niness, 1999; Saggu *et al*., 2014; Keshk *et al*., 2019).

However, some researches have informed that HAPNs displayed major cytotoxicity to some kinds of cancer cells like breast cancer cells (Meena *et al*., 2012), osteosarcoma cells (Qing *et al*., 2012), gastric cancer cells (Chen *et al*., 2007) and glioma cells (Xu *et al*., 2012). Therefore, the current study were performed to study the therapeutic effects of chicory extract against hydroxyapatite nanoparticles induced rat kidney damage by decreasing kidney functions, electrolytes, cytokines and apoptosis.

MATERIALS AND METHODS

Chemicals

Hydroxyapatite nanoparticles

Hydroxyapatite nanoparticles (HAP NPs) and the dose of hydroxyapatite nanoparticles was 300 mg/kg BW (dissolved in distilled water) >30 nm particle size were gotten from nano-tech Company (Nano-tech Egypt for Photo-Electronics, sales@nanotecheg.com).

Extract preparation

The extraction system of *Cichorium intybus* roots was finished as point by point by Saggu *et al*. (2014).

Animals

Healthy 40 male Wistar rats (weighting 160-170 gram and 13±1 weeks age), supplied from the Accredited Breeding and Experimental Laboratory in Faculty of Medicine, Alexandria University, Egypt.

The rats remained preserved on a standard rodent diet and water available *ad libitum*. The temperature in the animal room was maintained at 23±2^{o}C with a relative humidity of 55±5%. Light was on a 12:12 hr light - dark cycle. 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).

Experimental design

After two weeks of acclimation, animals were divided into 4 equal groups (10 rats per group):

Group 1 (Control): Negative control; as negative controls, these rats were untreated.

Group 2 (Chicory): Negative control receiving chicory; (20 mg/kg b.wt.) administered by oral gavage for 4 weeks (Saggu *et al*., 2014).

Group 3 (HAP NPs): Rats were injected intraperitoneally with HAP NPs (IP; 300 mg/kg BW /day) for 4 weeks.

Group 4 (HAP NPs+Chicory): treated of HAP NPs for 4 weeks and then treated with chicory extract for 4 weeks.

Tissue preparation

Rodents from individually gathering were euthanized with sodium pentobarbital and exposed to a total necropsy following 10-12 hours fasting (Salama *et al*., 2015). Blood tests from each rodent were pulled back from vena-cava and accumulated in non-heparinised tubes before being absent for thirty minutes to cluster at room temperature preceding being dependent upon a 5000 rpm divergent for ten minutes.

Electrolytes and kidney functions biomarkers

Creatinine and urea were estimated in sera according to Tousson *et al*. (2016) and Eldaim *et al*. (2019) respectively. Cystatin C in sere was estimated according to the method of Tizon *et al*. (2010). The method offered by Abd Eldaim *et al*. (2019) was used to estimate electrolytes levels (ions of Potassium, sodium, and calcium) in the sera consuming commercial tools (Sensa-core electrolyte, Andhra Pradesh, India).

Histopathological examination

Fixed kidney in 10% buffer neutral formalin were prepared for paraffin sectioning and subjected to histopathological examination using haematoxylin and eosin stains according to Tousson (2016).

Immunohistochemical detection

Expression of pro apoptotic Bax immunoreactivities (Bax-ir), TNFα-ir and PCNA-ir in rat kidney sections were identified using ABC (Avidin Biotin Complex) after El-Atrsh *et al*. (2019), Calabrese *et al*. (2004) and Tousson *et al*. (2011) respectively.

STATISTICAL ANALYSIS

Data were reported as mean values ± SE and one way ANOVA was used to detect significant differences between treatment groups. For biochemical results, the analysis was done by using the Statistical Package for the Social Sciences (SPSS software version 16), the criterion for statistical significance was set at $p<0.01$.

RESULTS

Markers of kidney damage

Relative to control and chicory groups, the HAP NPs group displayed significantly ($P<0.01$) elevated levels of serum urea, creatinine, cystatin C, potassium ions, and chloride ions (table 1), but significantly ($P<0.01$) decreased levels of serum sodium and calcium ions (table 1). By contrast, improvement in alterations in electrolytes,

cystatin C and kidney functions levels were detected after the post treatment of HAP NPs with chicory (HAP NP+ chicory) compared with HAP NPs group (table 1).

Effect of chicory on the kidney histopathology

Kidney sections in the control and chicory groups revealed normal histological structures of the glomeruli and tubules in the medullary and cortical portions (figs. 1A&1B). Kidney section in the treated rats with HAP NPs revealed severe hypertrophied cells lining the tubules of tubular cells and atrophied glomeruli, moderate necrotic tubular cells, with inflammatory cellular infiltration were observed (Fig. 1C). Moreover, kidney sections in the treated HAP NPs with chicory extract (HAP NPs+chicory) greatly improved the glomeruli and renal tubules (fig. 1D).

Changes of pro-apoptotic Bax protein expression in kidney

Kidney sections in HAP NPs showed moderate to strong

Table 1: Effect of hydroxyapatite nanoparticles and/or chicory extract on the serum of kidney functions, Cystatin C and electrolytes

Item	Control	chicory	HAP NPs	HAP NPs+ chicory
Urea (mg/dl)	$26.5^{\#} \pm 1.27$	$22.8^{\#} \pm 0.93$	$37.5^{*} \pm 1.63$	$27.0^{*} \pm 1.00$
Creatnine (mg/dl)	$0.44^{\#} \pm 0.04$	$0.41^{\#} \pm 0.03$	$0.73^{*} \pm 0.04$	$0.55^{\#} \pm 0.04$
Cystatin C (Pg/ml)	$7.65^{\#} \pm 0249$	$7.15^{\#} \pm 0.255$	$21.18^{*} \pm 1.262$	$9.49^{\#} \pm 0.854$
Na^{+} (mmol/l)	$136.6^{\#} \pm 11.13$	$135.5^{\#} \pm 9.97$	$121.6^{*} \pm 8.35$	$130.0^{\#*} \pm 11.05$
K^{+} (mmol/l)	$4.12^{\#} \pm 0.530$	$3.91^{\#} \pm 0.255$	$5.16^{*} \pm 0.495$	$4.70^{\#*} \pm 0.338$
Ca^{++} (mmol/l)	$1.183^{\#} \pm 0.07$	$1.206^{\#} \pm 0.13$	$0.885^{*} \pm 0.028$	$1.149^{\#*} \pm 0.024$
Cl^{-} (mmol/l)	$101.9^{\#} \pm 7.56$	$100.5^{\#} \pm 6.80$	$119.5^{*} \pm 8.75$	$108.0^{\#*} \pm 7.20$

Data are expressed as mean ± SE of 5 observations. #Significantly different from hydroxyapatite nanoparticles, *Significantly different from control at $p<0.01$.

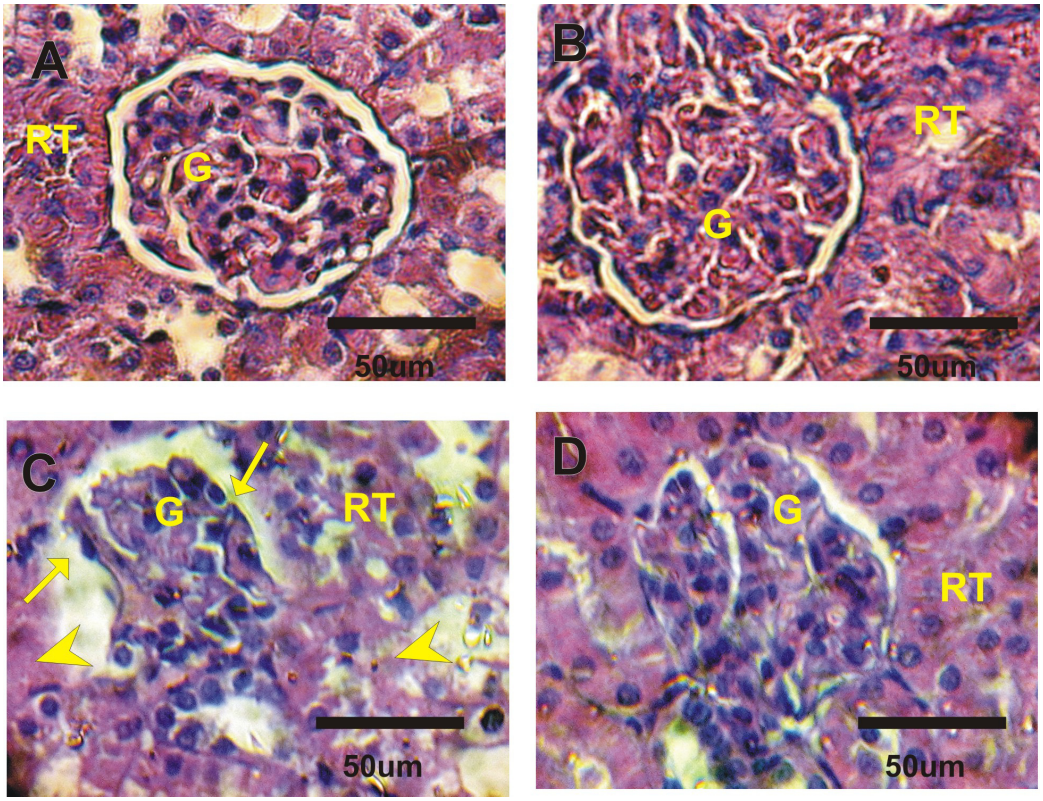

Fig. 1: Photomicrographs of Kidney sections in the different experimental groups stained with Hematoxylin & Eosin. A&B: Kidney sections in the control and chicory groups showed normal histological structures of the glomeruli (G) and tubules (RT). C: Kidney section in the treated rats with HAP NPs (G3) revealed hypertrophied cells lining the tubules, and atrophied glomeruli (arrows), moderate necrotic tubular cells (arrow heads), with inflammatory cellular infiltration. D: Kidney section in treated HAP NPs with chicory extract (HAP NPs+chicory) revealed a good degree of improvement in the glomeruli and renal tubules.

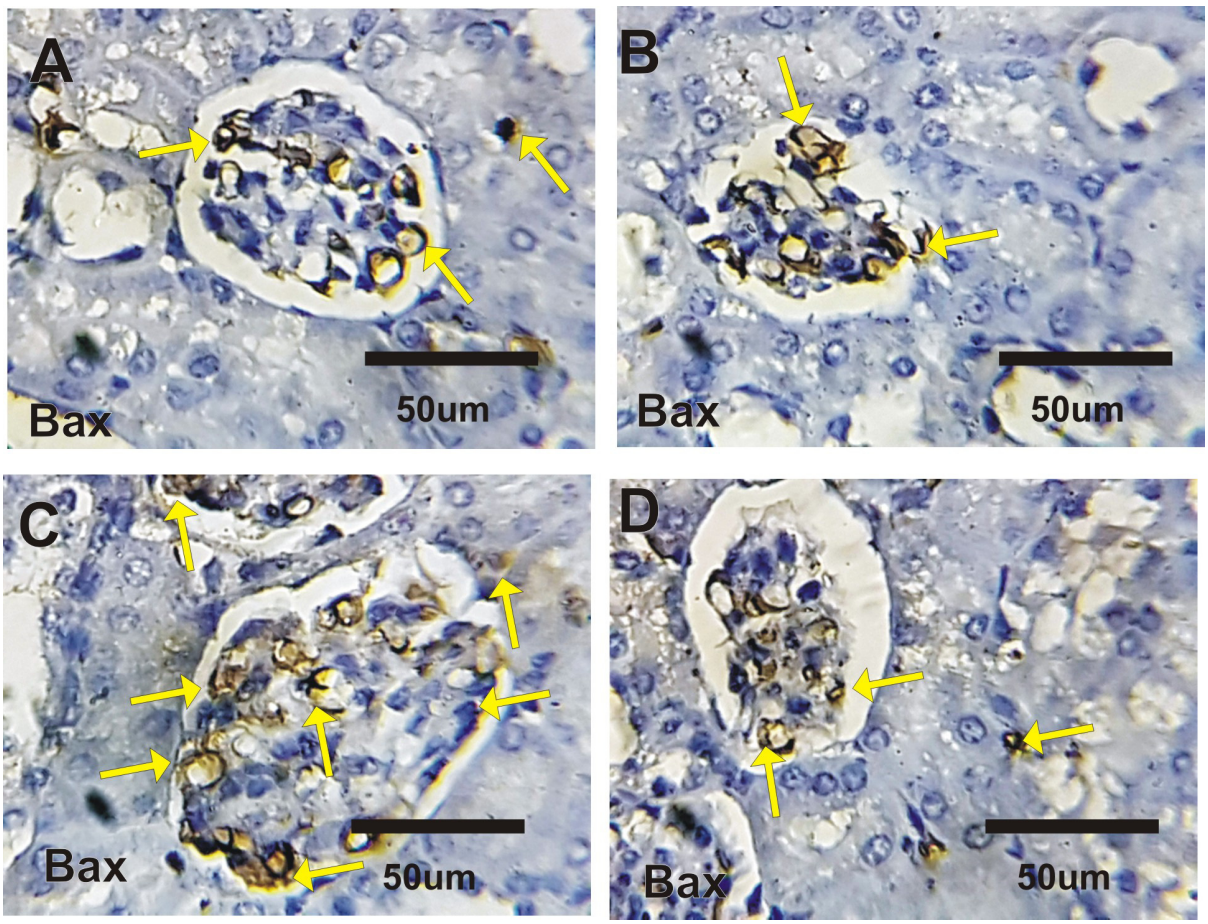

Fig. 2: Photomicrographs of kidney sections in the different experimental groups stained with Bax-ir. A&B: Mild positive reaction for Bax-ir in glomeruli (G) and renal tubules in control and chicory groups. C: Moderate positive reactions (arrows) for Bax-ir in HAP NPs group. D: Mild positive reactions for Bax-ir in HAP NPs+chicory group.

positive reaction for Bax expression relative to its expression in control and chicory groups that showed mild positive reaction (Figs. 2A-2C). Furthermore; kidney sections in the treated rats with HAP NPs with chicory extract (HAP NPs+chicory) revealed mild positive expression for Bax as compared to HAP NPs (fig. 2D).

Changes of TNFα expression in kidney

Kidney sections in the treated rats with HAP NPs showed moderate positive reaction for TNFα expression as compared to faint positive reaction in the control and chicory groups (figs. 3A-3C). Mild positive expressions for TNFα were observed in the kidney sections section in the treated rats with HAP NPs with chicory extract (HAP NPs+chicory) when compared to HAP NPs (fig. 3D).

Changes of PCNA expression in kidney

Kidney sections in the treated rats with HAP NPs showed moderate positive reaction for PCNA expression as compared to faint positive reaction in the control and chicory groups (Figs. 4A-4C). Mild positive expressions for PCNA were detected in the kidney sections section in the treated rats with HAP NPs with chicory extract (HAP NPs+chicory) when compared to HAP NPs (fig. 4D).

DISCUSSION

Nanoparticles can enter the human body via ingestion, inhalation, or skin contact and induced cardiovascular, respiratory, lymphatic, autoimmune, hepatic, reproductive and renal diseases that can manifest immediately following exposure or many years later. Relatively insufficient studies have been done to study the influence of hydroxyapatite nanoparticles (HAP-NPs) on the kidney functions and structure. Recently, role of natural antioxidant plants on renal toxicity has been studied by pharmacological researches (Tousson *et al*., 2016; Almakhatreh *et al*., 2019; Mutar *et al*., 2020). Along these lines, this study was planned to study the function of chicory extract in improving renal toxicity by hydroxyapatite nanoparticles (HAP NPs) in male rats.

Urea is a waste item framed from the breakdown of proteins while creatinine is a waste item made by the

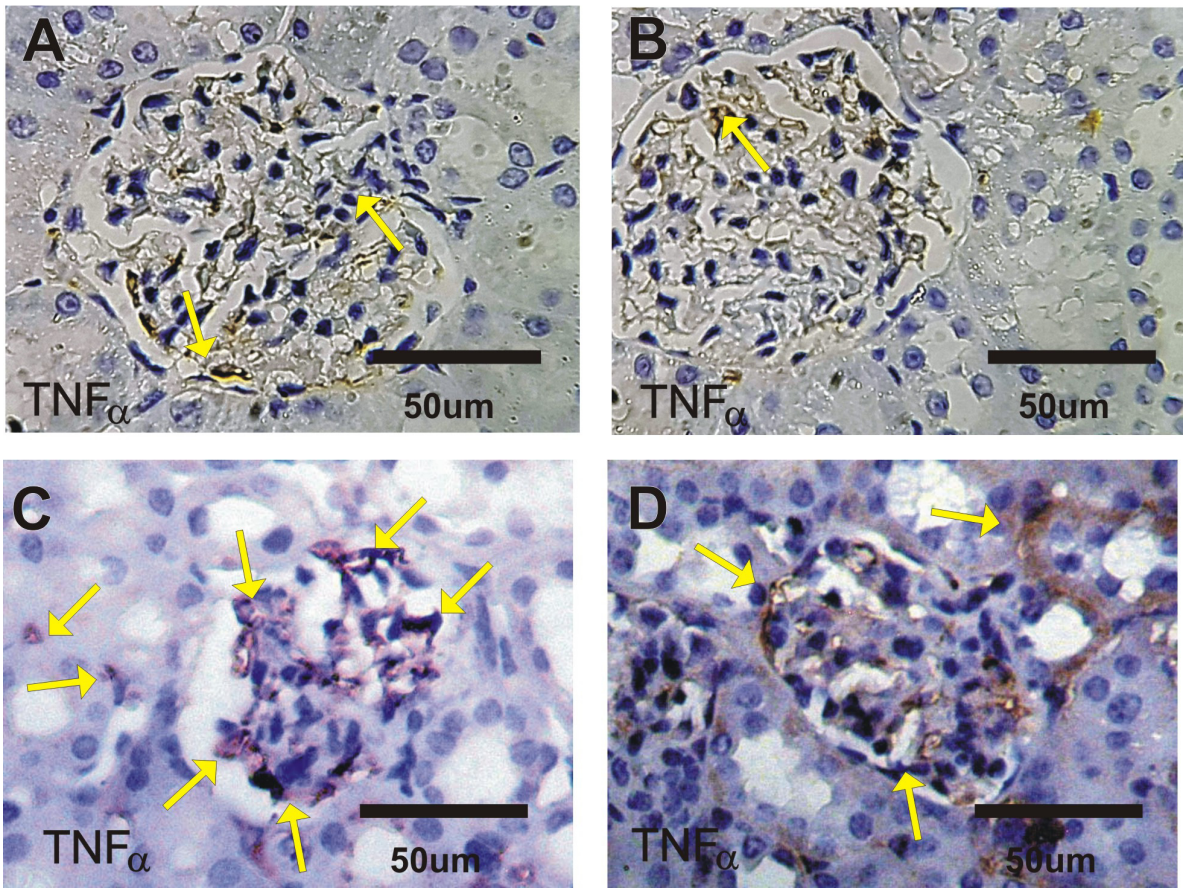

Fig. 3: Photomicrographs of kidney sections in the different experimental groups stained with TNFα-ir. A&B: Faint positive reaction for TNFα-ir in glomeruli (G) and tubules in control, and chicory groups. C: Moderate positive reactions for TNFα in HAP NPs group. D: Mild positive reactions for TNFα in HAP NPs+chicory group.

muscles. Serum urea and creatinine are helpful records for assessing the status of renal capacity and the creatinine levels is typically a more exact marker of kidney work than urea (Tousson *et al*., 2014). The elevation serum urea levels may suggest debilitated renal discharge (Alm-Eldeen and Tousson, 2012; Barakat *et al*., 2015). Iavicoli *et al*. (2016) who reported that; nanoparticles induced renal toxicity. Current results revealed that; treated rats with HAP NPs induced significant elevation in the levels of creatinine, urea, cystatin C, potassium ions and chloride ions, ($P < 0.01$) by comparison to the control and chicory groups. In contrast; decline in serum sodium and calcium ions were observed after the treatments of rats with HAP NPs. Post treatments of rats with HAP NPs and chicory extract (HAP NPs+chicory) revealed improved the kidney functions and structure. This elevation in kidney functions is due to severe atrophy in glomeruli and renal tubules, necrotic cells, and mild inflammatory cellular infiltration as confirmed by histological result. Our results align with Mosa *et al*. (2019) who stated that hydroxyapatite nanoparticles induced renal toxicity, damage in DNA, oxidative stress, and changes in histological and immunohistochemical reactions in male rats. Our results in line of Tang *et al*. (2010), Guo *et al*. (2009) who find that; TiO2NPs have elevated the levels of urea creatinine and uric acid. This increase in potassium ions and the decrease in sodium ions levels may be due to kidney injury. In kidney injury outcome is decreasing in GFR and tubular flow, also tissue injury and acute K^+ load are occurred. All these factor causes hyperkalaemia in acute kidney injury (Kovesdy, 2014). Contradictory to our results, Liu *et al*. (2005) reported that; acute induction of hydroxyapatite nanoparticles on rabbits not induced renal toxicity and no changes in calcium.

Current results revealed; significant depletion in the levels of creatinine, urea, Cystatin C, potassium ions and chloride ions after the post treatment of rats with HAP NPs and chicory extract as compared to HAP NPs group. Wang *et al*. (2019) who reported that; chicory slows renal reabsorption by controlling expression of urate transporters in fructose-induced hyperuricemia. These data are supported by Jin *et al*. (2018) who studied the influences of chicory on serum kidney functions, and in vitro verification with cells and reported that; chicory

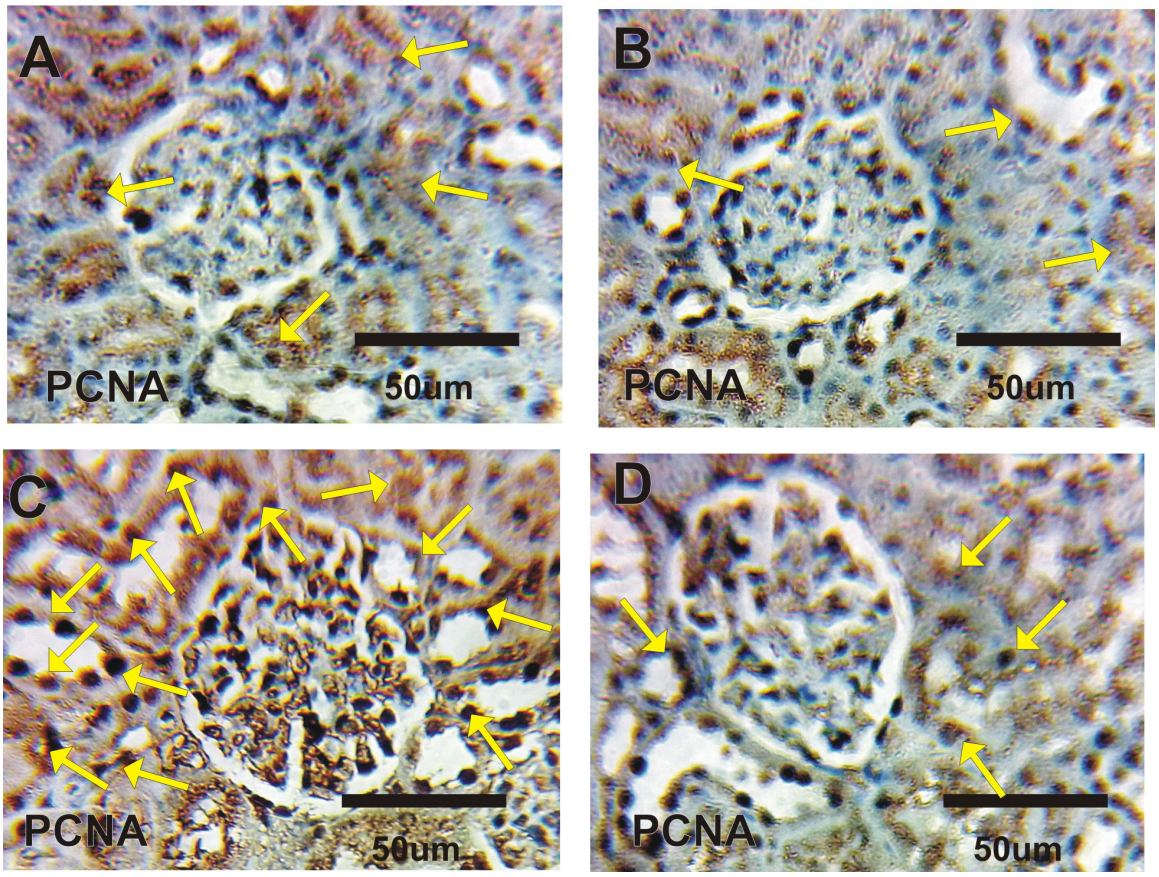

Fig. 4: Photomicrographs of kidney sections in the different experimental groups stained with PCNA-ir. A&B: Faint positive reaction for PCNA-ir in glomeruli (G) and renal tubules in control and chicory groups. C: Moderate positive reactions (arrows) for PCNA-ir in HAP NPs group. D: Mild positive reactions for PCNA-ir in HAP NPs+chicory group.

extract inhibits the elevation in urea, uric acid, and creatinine in hyperuricaemic rats with kidney damage. Also our results are in alignment with Pourfarjam *et al*. (2017) who find that; the extract of chicory seed decrease kidney functions parameters in experimentally induced early and late diabetes type 2 in rats.

Many studies reports a different harmful cellular effects including after nanoparticles exposure as oxidative stress, damage in the cellular DNA, tissue injury, and changes in gene expression (Singh *et al.,* 2010; Khanna *et al.,* 2015; Mosa *et al*., 2018; Altwaijry et al., 2020). The current study revealed that; injected rats with hydroxyapatite nanoparticles revealed renal injury with severe hypertrophied cells lining the tubules of tubular cells and atrophied glomeruli, moderate necrotic tubular cells, with inflammatory cellular infiltration. Also; an increase in the pro-apoptotic Bax protein, cytokines TNFα and proliferation PCNA expressions in kidney sections as compared to control. Current results agree with Mosa *et al*. (2019) who reported that hydroxyapatite nanoparticles induced renal inury, DNA damage, oxidative stress, and an increase in PCNA expression in male rats. These data are supported by Chen *et al*. (2006); Wang *et al*. (2008) reported that; many types of nanoparticles administration induced toxic effects mainly on liver, kidneys, and spleen tissues. Also; Esmaeillou *et al*. (2013) who found that; ZnO nanoparticles induced kidney toxicity and injury in healthy adult mice. Our results agree with Wang *et al*. (2008) who find that; ZnO nanoparticles demonstrated induced severe renal toxicological impact and renal damage. Also our results are in alignment with Pourfarjam *et al*. (2017) who reported that; *Cichorium intybus* L. seed extract is capable of reversing kidney damage based on its ability to substantially decrease urinary α1-microglobulin and enhance the histological appearance. Also; Gui *et al*. (2013) who find that; titanium dioxide nanoparticles induced nephrotoxicity in mice.

Nanoparticles can up-regulate the transcription of many pro-inflammatory genes, like tumor necrosis factor-α (TNF- α) and interleukins, through signaling activation of nuclear factor-kappa B (Khanna *et al*., 2015). These molecular and cellular actions can cause oxidative stress, then severe cellular genotoxicity and subsequently

apoptosis (Raafat *et al*., 2011; Taha *et al*., 2018; El-Atrsh *et al*., 2019). Apoptosis and necrosis are two characteristic types of cell death, which are discussed in many reports. TNFα is type of pro-inflammatory cytokine. TNF-α concentration increases in renal failure, glomerulonephritis, diabetic nephropathy, and interstitial tubular nephritis (Ramseyer and Garvin, 2013). In our study, we evaluated the apoptosis, for proliferating cell nuclear antigen (PCNA) and cytokines occurring in kidney section after injection with hydroxyapatite nanoparticles. Also; post treatment of hydroxyapatite nanoparticles with chicory (HAP NPs+chicory) improved the renal damage, elevation in Bax, PCNA and TNF-α expression as compared to injected rats with hydroxyapatite nanoparticles. Our outcomes agree with Mahmoudian *et al*. (2016) who informed that; silver nanoparticle intraorally administration induced injury and apoptosis in rat liver. Supportive with our results, Pujalté *et al*. (2011) reported that; metallic nanoparticles induced kidney cytotoxicity, oxidative stress and apoptosis. These data are supported by Park *et al.* (2010) reported that oral administration of silver NPs induced elevation in TNF-α and IL-6.

CONCLUSIONS

Hydroxyapatite nanoparticles (HAP NPs) induced renal toxicity, tissue injury, changes in electrolytes levels, apoptosis, cytokines and proliferation alterations. Chicory extract may offer advantages against the dangerous nature of hydroxyapatite nanoparticles.

ACKNOWLEDGEMENT

This work was funded by DSR (Deanship of Scientific Research), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia, under grant RGP-1440-0022. The authors therefore, gratefully acknowledge the DSR technical and financial support.

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