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Histological and molecular characterization of the protective effect of *Eugenia caryophyllata* against renal toxicity induced by vitamin D in male wistar rats

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Abstract

Vitamin D can be harmful if taken for a long time, or taken in large acute dose, cause many problems including hypercalcemia. Here, we examined the protective effects of *Eugenia Caryophyllata* against vitamin D toxicity induced renal inflammation in rats. This study employed four experimental groups ($n = 10$) that underwent two months of treatment as follows: control group animal received saline (0.9% NaCl), vitamin D group that was received (100 mg/kg) of vitamin D, Eugenol group that was received Eugenol (100 mg/kg), vitamin D + eugenol group that was received vitamin D (100 mg/kg) then will be orally given Eugenol (100 mg/kg). The current study showed that a significant upregulation in the IL-2, TNF- α and iNOS gene expression levels caused by vitamin D compared to the control group. Eugenol treatment caused a significant decreased IL-2, TNF-α and iNOS activity and also upregulated gene expression levels. Histological features investigated showed that administered with vitamin D intoxication showed distinct alterations from that of the untreated control groups, such Shrinkage of glomeruli, hypercellularity of the glomeruli and necrosis. However, retreatment with Eugenol showed improvement in the histological features in by slight infiltration, fibrosis, minimal pleomorphism and less disarrangement and compared with that of Vitamin D treated rats. In conclusion, the Eugenol exhibited renoprotective effects against vitamin D-induced nephrotoxicity. These renoprotective effects could be achieved by the antioxidant and anti-inflammatory activities of the Eugenol. Thus, for vitamin D-induced nephrotoxicity, the use of Eugenol could be beneficial.

Keywords: vitamin D; Eugenol; hypercalcemia; kidney; inflammatory biomarkers; glomerulonephritis.

Practical Application: Eugenol attenuates vitamin D-induced nephrotoxicity accomplished by regulating oxidative stress.

1 Introduction

The term vitamin D (Calciferol) refers to a group of fat-soluble secosteroids with endocrine function. There are two major forms of vitamin D, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). There are studies suggesting that vitamin D_2 may be less potent than vitamin D_3 in the human body (Houghton & Vieth, 2006). It requires a protein carrier for solubility in plasma because it is a lipophilic molecule similar to its closely related lipid precursor cholesterol (Ready, 2021). It is stored in adipose tissue. It has been suggested that vitamin D stores in adipose tissue may not be readily available when needed (Szymczak-Pajor et al., 2022). It has an anti-inflammatory and immune-modulating properties (Alshahrani & Aljohani, 2013). It is an important pro-hormone, which plays an essential role in the regulation of calcium and phosphorus absorption metabolism for bone homeostasis (DeLuca, 2004; Bringhurst et al., 2010). Moreover, vitamin D is increasingly recognized to have beneficial effects in several inflammatory conditions, and there is some evidence to suggest that it is associated with a reduced risk of various internal malignancies. Vitamin D have a broader role in maintaining health, including protection against autoimmunity, infection, and cancer (Aranow, 2011; Peterlik et al., 2013). Both vitamin D_3 and vitamin D_2 can be activated by undergo the same process (Jones et al., 1998). They are both inactive prohormones that bind to the vitamin D–binding protein (DBP) to be transported (Braegger et al., 2013). In its native form, vitamin D is not biologically active, and it activated to $1,25(OH)_2D$. The safe upper limit of vitamin D dosage for children may be reached to 2,000 IU of vitamin D/day, and for adults, up to 10,000 IU of vitamin D/day has been shown to be safe (Holick, 2006). For many people the word "vitamin" implies something that is beneficial, essential and not potentially poisonous but unfortunately vitamin D is toxic in large doses (Bischoff-Ferrari et al., 2005; Lotfollahi et al., 2021). High doses of vitamin D have been known for many years to be toxic to humans, rats and other animals. In humans, manifestations of vitamin D toxicity include hypercalcemia, hypercalciuria, nausea, anorexia, lethargy, mental disturbances, ectopic soft tissue calcification, including vascular calcification and nephrocalcinosis, and renal failure (Coburn & Jack, 1984; Pettifor et al., 1995).

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The mechanisms behind this renal concentration defect are incompletely understood and may involve tubular interstitial injury because of down-regulation of aquaporin-2 water channel, or activation of the calcium-sensing receptors or due to calcium deposition in the medulla (Hebert, 1996; Sands et al., 1997). Causes damage to cells followed by calcium deposition in tubules, basement membrane and loop of Henle which lead to nephrocalcinosis (Beşbaş et al., 1989; Sibernagl & Lang, 2000). Kidney function is affected because hypercalcemia alerts the action of vasopressin on the renal tubules. The net result is reduced urinary concentrating ability and a form of nephrogenic diabetes insipidus. This usually presents as polyuria, but rarely is the volume as high as that association with central diabetes insipidus (Marcinowska-Suchowierska et al., 2016).

Synzygium aromaticum (clove) is one of the most important herbal medicines that have been used in a wide range in food spices, preservatives and many medical proposes (Cortés-Rojas et al., 2014). It contains volatile oil used as nervous stimulants and cognitive enhancer (Halder et al., 2011). The major chemical constituents in clove called polyphenylpropene eugenol, which gives the clove its specific flavour. Several studies reported the beneficial effect of eugenol (*in-vitro and in-vivo*) as antiproliferative, anti-infalmmatory, cytotoxic and antioxidant compounds (Said & Rabo, 2017).

The present work was aimed to evaluate the protective effects of Eugenol against vitamin D – induced toxicity in rat's kidney by using light microscopes, bioassay studies and molecular biology in young male Wistar rat and these will be via examine the histological changes which probably induced in kidney and evaluate the protective effect of Eugenol against vitamin D toxicity at the level of some genes in kidney of rat.

2 Materials and methods

2.1 Drugs used

Vitamin D purchased from Novartis Company.

Eugenol purchased from Sigma-Aldrich Company.

2.2 Experimental animals

Forty young healthy male Westar rats weighing about 200 gm were obtained from Animal House King Saud University .Animals were housed in plastic cages under controlled temperature (23 ± 28 °C), and maintained in groups of five per cage in a light-dark cycle .They were given free access to a commercial pellet diet and tap water, and allowed to acclimatize for three days before initiation of the experiment. They were divided into four groups (10 animals each) and will be treated as follows

Group I: control group (-ve) each animal orally given saline (100 mg/kg b/w) for 2 months .

Group II: control group (+ve) each animal orally given vitamin D (100 mg/kg /w) for 2 months (Holcombe et al., 2015)

Group III: Each animal orally given Eugenol (100 mg/ kg b/w) for 2 months (Paula-Freire et al., 2016)

Group IV: Each animal orally given vitamin D (100 mg / kg b/w) then orally given Eugenol (100 mg / kg b/w) for 2 months.

2.3 Total phenolic content

Total phenolic compound content of *G.* **Eugenol** extract was assayed by the Folin-Ciocalteu method as described previously (Moneim, 2013). Briefly, 0.1 mL of the sample's extract was mixed with 2.5 mL of distilled water in a test tube, and then 0.1 mL of undiluted Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) was added. The solution was mixed well and then allowed to stand for 6 min before adding 0.5 mL of 20% sodium carbonate solution. The color developed for 30 min at room (20 °C) temperature and the absorbance was measured at 760 nm using a spectrophotometer (PD 303 UV spectrophotometer, Apel Co., Limited, Saitama, Japan). A blank sample was prepared using 0.1 mL of methanol instead of the extract. The measurement was compared to a calibration curve of gallic acid solution and expressed as milligram (mg) equivalent (eq.) of gallic acid per gram (g) of dry weight extract.

2.4 Total flavonoids

The aluminum chloride colorimetric method was used to determine the total flavonoid content of Eugenol extract as described previously (Akillioglu & Karakaya, 2010). Briefly, in a test tube, 50 µL of the extract was mixed with 4 mL of distilled water, 0.3 mL of 5% $NaNO_2$ solution, and 0.3 mL of 10% $AlCl_3.6H_2O$. The mixture was allowed to stand for 6 min and then 2 mL of 1 mol/L NaOH solution was added; distilled water was subsequently added to bring the final volume to 10 mL. The mixture was allowed to stand for another 15 min and the absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve and the result is expressed as mg eq. rutin per g dry weight.

2.5 DPPH (2,2–diphenyl–1–picrylhydrazyl) Scavenging Activity Radical

The power of the **Eugenol** extract to scavenge DPPH radicals was assayed as described previously (Akillioglu & Karakaya, 2010). A fresh solution of 0.08 mM DPPH radical in methanol was prepared. Next, 950 µL of DPPH solution was mixed with 50 µL extract and incubated for 5 min. Exactly 5 min later, the absorbance of the mixture was measured at 515 nm (PD 303 UV spectrophotometer, Apel Co., Limited). Antioxidant activity (AA) is expressed as percentage inhibition of DPPH radical using the equation below; $AA = 100 - [100 \times (A_{sample}/A_{control})]$, where A_{sample} is the absorbance of the sample at time, t = 5 min and $A_{control}$ is the absorbance of the control.

2.6 ABTS [2,4,6–tri(2–pyridyl)–s–triazine] Radical Scavenging Activity

The ABTS assay was used to determine the DPPH radical scavenging activity according to the method of Gouveia & Castilho (2011). The $ABTS^{\cdot+}$ radical solution was prepared by reacting 50 mL of 2 mM ABTS solution with 200 µL of 70 mM

potassium persulfate solution. This mixture was stored in the dark for 16 h at room temperature and it was stable in this form for two days. For each analysis, the $ABTS^{\cdot+}$ solution was diluted with pH 7.4 phosphate buffered saline (PBS) solution to an initial absorbance of 0.700±0.021 at 734 nm.

This solution was freshly prepared for each set of analysis. To determine the antiradical scavenging activity, an aliquot of 100 µL methanolic solution was mixed with 1.8 mL of $ABTS^{\cdot+}$ solution and the decrease in absorbance at 734 nm (PD 303 UV spectrophotometer, Apel Co., Limited, Saitama, Japan) was recorded during 6 min. The results are expressed as µmol Trolox equivalent per g of dried extract (µmol eq. Trolox/g), based on the Trolox calibration curve.

2.7 Ferric Reducing Antioxidant Power (FRAP)

Ferric reducing antioxidant power (FRAP) was performed as described previously (Benzie & Szeto, 1999). The FRAP reagent included 300 mM acetate buffer, pH 3.6, 10 mM 2,4,6–Tris(2–pyridyl)–s–triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3$ in the ratio 10:1:1 (v/v/v). A volume of 3 mL of the FRAP reagent was mixed with 100 L of moringa extract in a test tube and incubated with shacking at 37 °C for 30 min in a water bath. Reduction of ferric–TPTZ to the ferrous complex formed an intense blue color, which was measured with a UV–visible spectrophotometer (PD 303 UV spectrophotometer, Apel Co., Limited) at 593 nm after 4 min. The results are expressed in terms of mol eq. Trolox per g of dried sample (µmol eq. Trolox/g).

2.8 Real Time PCR

The total RNA was isolated from the kidney tissue using an RN easy plus Minikit (Qiagen,Valencia,CA).One microgram of the total RNA and random primers were used for cDNA synthesis using the Revert Aid H minus ReverseTranscriptase(Fermentas,ThermoFisherScientificInc.,Canada). For real time PCR analysis, the cDNA samples were run in triplicate and GAPDH was used as a reference gene. Each PCR amplification included non-template controls and all reagents except for the cDNA. Real time PCR reactions were performed using Power SYBR Green(Life Technologies, CA) and were conducted on the Applied Biosystems 7500 Instrument. The typical thermal profile is 95 °C for 3min, followed by 40 cycles of 95◦C for 15s and 56 °C for 30s. After PCR amplification, the 1Ct was calculated by subtracting the GAPDH Ct from each sample Ct. The method of Pfaffl was used for the data analysis (Pfaffl, 2001). The PCR primers for iNOS, IL-2 and TNF-α genes were synthesized by Jena Bioscience GmbH(Jena, Germany). Primers were designed using the Primer-Blast program from NCBI. For a reference gene, GAPDH was used.

The primer sets used were as the following:

GAPDH: Sense: 5'-GCATCTTCTTGTGCAGTGCC-3',

Antisense: 5'-GATGGTGATGGGTTTCCCGT-3';

iNOS: Sense:5'-GTTCCTCAGGCTTGGGTCTT-3',

Antisense:5'- TGGGGGAACACAGTAATGGC-3';

IL-2: Sense: 5'-CTGCAGCGTGTGTTGGATTT-3',

Antisense: 5'-GGCTCATCATCGAATTGGCAC-3';

TNF-α: Sense: 5'-AGAACTCAGCGAGGACACCAA-3',

Antisense: 5'-GCTTGGTGGTTTGCTACGAC-3'

2.9 Histopathological studies

Kidney tissue samples were fixed in 10% neutral formalin for 24h and paraffin blocks were routinely processed for light microscopy. Slices of 4–5 µm were obtained from the prepared blocks and stained with hematoxylin and eosin as well as Masson's trichrome for hepatic fibrosis. The preparations obtained were visualized using a Nikon microscope.

2.10 Statistical analysis

Results were expressed as Mean ± SE (standard error). Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (SPSS version 22).

3 Results

The total amounts of the phenolic and flavonoids contents present in the Eugenol were 12545.203 ± 163.740 mg eq. gallic acid/g and 454.906 ± 8.878 mg eq. rutin/g, respectively Table 1. Furthermore, the results indicated that the Eugenol has potent free radical scavenging power. For the DPPH, ABTS, and FRAP assays, values of 76.206 ± 0.268, 2466.667 ± 11.547 and 2321.566 ± 92.459 µmol eq. Trolox/g, respectively, were obtained.

Histopathological patterns showed in the kidney (Figure 1): The results of examination showed that normal structure in the control group, examination of Eugenol and vitamin D-treated rat kidney revealed that treatment with Eugenol was able to ameliorate the vitamin D-induced kidney toxicity. While Vitamin D-administration produced many alterations in the renal tissue, showing massive degeneration and hypercellularity of glomerulus.

During inflammation, nucleus had a role in immunity, because it activates pro-inflammatory genes encoding *iNOS*, *TNF-α*, and *IL-2*.The current study showed a significant up regulation in gene expression of *iNOS* mRNA, *TNF-α* mRNA and IL-*2* mRNA which induced by Vitamin D overdose compared to control group. In contrast to our data, it has been reported that, the treatment with Eugenol group caused significant decreased and downregulated gene expression levels of *IL-2* mRNA, *iNOS* mRNA and *TNF-α* mRNA (Figure 2).

Table 1. Experimental determination of total phenolic and flavonoids contents and antioxidant capacity assays (ABTS, DPPH, and FRAP) for the Eugenol.

Parameters	Mean ± SD
Total phenols (mg eq. Gallic acid/g sample)	12545.203 ± 163.740
Total flavonoids (mg eq. Rutin/g sample)	454.906 ± 8.878
DPPH (%)	76.206 ± 0.268
ABTS (µmol eq. Trolox/g sample)	2466.667 ± 11.547
FRAP (µmol eq. Trolox/g sample)	2321.566 ± 92.459

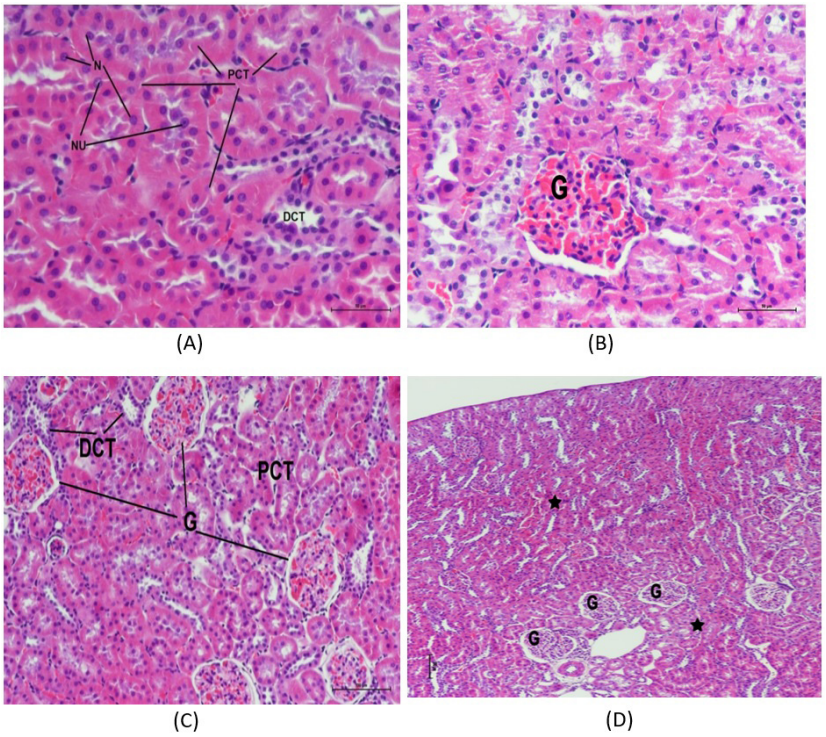

Figure 1. Effect of Eugenol on the histopathological damage induced by Vitamin D on the kidneys of rats Kidney sections [specimen fixed in 10% neutral buffered formalin, and stained with Hematoxylin and Eosin (H&E)]. **(A): Group 1,** control rats without any signs of kidney damage, showing proximal convoluted tubules (PCT) with euchromatic nuclei (N) and prominent nucleolus (Nu). **(B): Group I1,** showing massive degeneration of renal tubules and hypercelllarity of glomerulus (G), luminal capillaries of glomerulus filled with RBCs. **(C): Group I1I,** showing regular histological features of glomeruli (G) more or less similar to control, proximal convoluted tubules (PCT), and distal convoluted tubules (DCT). **(D)**: **Group IV,** showing the normal distribution of glomeruli (G) and renal tubules (star) of the cortex. 400X

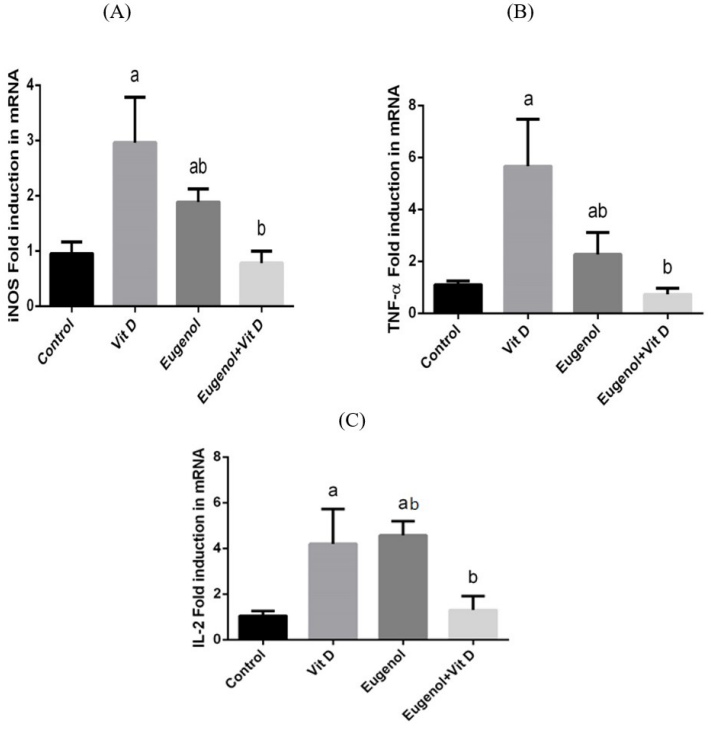

Figure 2. Effect of Eugenol on renal gene expression of IL-2 mRNA, iNOS mRNA and TNF-α mRNA induced by Vitamin D toxicity (100 mg / kg.b.w.). Values are means ± SEM (n=10) [a].significant change at $p < 0.05$ with respect to the negative control group .[b] a.significant change at $p < 0.05$ with respect to the positive group.

4 Discussion

Plant medicine has been used for more than 5,000 years. The interest in polyphenols has grown considerably because of their high capacity to trap free radicals associated with different diseases. Phenols and flavonoids are very important plant constituents because of their antioxidant activity. The plant phenolics are commonly present in fruits, vegetables, leaves, nuts, seeds, barks, roots and in other plant parts (Kaviarasan et al., 2007; Kaba, 2017). The antioxidant activity of phenolic compounds is mainly due to their redox properties which play an important role as free radical scavengers, reducing agents, quenchers of singlet oxygen and complexes of pro-oxidant metals (Mustafa et al., 2010).

Oxidative stress results from the imbalance of reactive oxygen species (ROS) and defense mechanisms, which results in cell damage. In addition, the presence of inflammation is a well-documented factor influencing the development of oxidative stress in dialysis patients (Samouilidou et al., 2003; Sies et al., 2017). Renal sources for ROS are activated macrophages, vascular cells and various glomerular cells. ROS may affect cells of the host organism, especially at sites of inflammation, in addition to playing a role in the defense system against other agents. This effect plays a role in a variety of renal diseases such as glomerulonephritis and tubulointerstitial nephritis, which can contribute to proteinuria and other conditions (Ratliff et al., 2016; AlYousef et al., 2020). This suggests that the kidney may be particularly susceptible to oxidative stress.

Vitamin D in high dose cause nephrotoxicity extensively used to induce oxidative stress in laboratory animals. Its mode of action is based on the propagation of the lipid peroxidation of the membranous system and depletion of antioxidant status and DNA injuries in the kidneys of rats (Khan et al., 2010; Amrein et al., 2014). Development of a renal disease is the result of multiple processes. Experimental and clinical results indicate that oxidative stress may be the link connecting different types of chronic renal injuries (Moneim et al., 2011; Al-Olayan et al., 2014). Previous reports suggest that vitamin D is the best-characterized tool for the study of oxidative stress trials as it consistently generates free radicals with the implication of a pathological environment by damaging the integrity of cell membranes and affecting physical parameters of the kidney such as the urinary and serum profiles (Özkan et al., 2012).

Kumar & Pandey (2013) reported that the alterations induced by vitamin D in the kidney function, were characterized by signs of injury such as changes in kidney tissue. The present study showed that administration of vitamin D to rats cause a reduction in the glomerular filtration rate., which induce mesangial cell contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that decrease the glomerular filtration rate (Nasri & Mubarak, 2013).

Plants have been used for medicinal purposes since time immemorial (Matsumura et al., 2000). Various studies have been documented immunomodulatory effect of essential oils and plant extracts including ginger, sage, clove oil, and tea oil (Golab et al., 2005; Carrasco et al., 2009).

The most common uses were in Europe, e.g. the use of pine oils against ectoparasites and for wound disinfection, camomile and yarrow to treat inflammations or anise, fennel and caraway fruits to prevent gastrointestinal problems, especially colic and flatulence (Zitterl & Franz, 1999).

Phytogenic components present in a variety of medicinal plants have been extensively used for the prevention and treatment of different lifestyle related risk factors. Traditionally, extracts of different parts of plants have been recommended to cure various complications including bronchitis, diarrhea, skin diseases, cancer, hyperlipidemia, liver ailments, hyperglycemia arthritis, cardiovascular diseases and inflammatory disturbances. The functionality of these plants is proposed due to the presence of a plethora of bioactive ingredients found in them (Prakash & Gupta, 2005).

Natural products containing bioactive phytochemicals are potentially important sources of anti-inflammatory drugs (Yogalakshmi et al., 2010), it is possessed stronger antioxidant activity which is likely to quench free radicals (Youdim et al., 1999). The antioxidants activity may act in various ways by scavenging the radicals, decomposing peroxides and chelating metal ions (Chaieb et al., 2007).

Clove (Syzygium aromaticum, syn. Eugenia aromaticum or Eugenia caryophyllata) is an aromatic dried bud of a tree from the family Myrtaceae, commonly used as a spice to add flavor to food preparations (Kim et al., 1998; Hemalatha et al., 2016).

Recently, there is no adequate therapy for vitamin D nephrotoxicity, there has been a growing interest in the use of antioxidants that can prevent vitamin D toxicity. Eugenol is a natural antioxidant that has a high content of phenolic compounds (Monti et al., 2017).

Eugenol can be obtained from a wide range of plant sources including cloves, basil, cinnamon and nutmeg (Kamatou et al., 2012). The clove buds and leaves are rich sources of Eugenol containing about 70–85% Eugenol (Mukherji, 1995). Pharmacological studies showed that Eugenol also has antibacterial, antifungal, antioxidant, anticancer, antipyretic, anti-inflammatory, and insect repellent activities (Kong et al., 2013). A wide range of pharmacological properties for Eugenol in nephrotoxicity, chronic inflammation, cancer, as well as metastasis were demonstrated (Said, 2011; Nam & Kim, 2013), the antioxidant activity of Eugenol associated with its phytochemicals, such as polyphenols, flavonoids and anthocyanidins, has gained importance (Karmakar et al., 2012). Gülçin (2011) demonstrated that the in vitro antioxidant ability of the Eugenol, rich in polyphenols and anthocyanidins, is also considered a powerful antioxidant. The antioxidants activity may act in various ways by scavenging the radicals, decomposing peroxides and chelating metal ions (Chaieb et al., 2007).

Our histological findings there was no abnormal appearance or histological changes in the kidney of control rats which were injected saline only or in Eugenol-treated rats, where there are normal proximal and distal tubules, while vitamin D administrated group caused classical damage in the rat kidney after 8 weeks by a shrunken glomeruli, inflammatory cellular infiltrations, cytoplasmic vacuolation and dilatation of some kidney tubules, the kidney tubules apparently contained more apoptotic cells as compared to the kidneys of the control rats.

While treatment with Eugenol markedly prevented the collapse of the glomeruli and largely prevented the vitamin D-induced histopathological changes in the renal tissue, and these agreement with (Nasri & Mubarak, 2013; Markakis et al., 2016).

NF-kB, a major transcription factor, modulates inflammatory system through expressing pro-inflammatory genes including inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) (Siebenlist et al., 1994; Ghosh et al., 1998). Under normal conditions, NF-kB is resident as an inactivation form complex with inhibitors of kB (IkB) in the cytoplasm. Once stimulated by inflammatory signals such as LPS and TNF-a, IkB is phosphorylated and degraded resulting in free NF-kB (Karin & Ben-Neriah, 2000; Lappas et al., 2002).

NF-κB regulates host inflammatory and immune responses and cellular growth properties (Barkett & Gilmore, 1999; Zhong et al., 2016) by increasing the expression of specific cellular genes. Cytokines that are stimulated by NF-κB, such as IL-1β and TNF-α, can also directly activate the NF-κB pathway, thus establishing a positive autoregulatory loop that can amplify the inflammatory response and increase the duration of chronic inflammation. NF-κB also stimulates the expression of enzymes whose products contribute to the pathogenesis of the inflammatory process, including the inducible form of nitric oxide synthase (iNOS), which generates nitric oxide (NO), and the inducible cyclooxygenase (COX-2), which generates prostanoids (Pahl, 1999; Kagoya et al., 2014).

In addition, NF-κB regulates IL-2 production, which increases the proliferation and differentiation of T lymphocytes (Gerondakis et al., 1998; Pahl, 1999). Thus, activation of NF-κB leads to the induction of multiple genes that regulate the immune and the inflammatory response.

Vitamin D injection for 8 months induces a cascade of inflammatory reactions and modulates the immune cells with increased production of proinflammatory cytokines, particularly TNF-α, IL-2 which accounts for further kidney damage.

TNF-α is a cytokine produced by activated macrophages in response to pathogens and other injurious stimuli, like xenobiotics, and is a necessary and sufficient factor for local and systemic inflammation. TNF-α amplifies and prolongs the inflammatory response by triggering other cells to release both cytokines, such as interleukin-2, and iNOS, all of which promote further inflammation and tissue injury (Guzik et al., 2003).

Our results are supported by the findings of Karim et al. (2013) who found that vitamin D toxicity significantly increased TNF-α, interleukin-2, and iNOS in rats. Furthermore, Zittermann et al. (2007) reported that vitamin D toxicity cause upregulation of renal TNF-α, interleukin-2, and iNOS. Indeed, it has been assumed that TNF-α, interleukin-2, and iNOS, are an important mediator in the development of vitamin D-induced toxicity. However, treatment of Eugenol downregulated the expression of TNF-α, interleukin-2 and iNOS compared to that of the vitamin D-induced group, demonstrating the antiinflammatory activity of Eugenol.

In contrast to our data, it has been reported that Eugenol treatment caused a significant decreased (iNOS) (IL-2), and (TNF a) gene and down-regulation gene expression levels. This is agreement with a previous report by (Huang et al., 2015).

The principal methoxyphenol of Eugenol has documented anti-inflammatory potential. Eugenol suppresses cyclooxygenase (COX)-2 expression and tumor necrosis factor (TNF) signaling, whereas Eugenol oligomers avert inflammatory cytokine expression in macrophages and NF-kappa B (nuclear factor-kappa B) activation (Magalhães et al., 2010). The anti-inflammatory mode of action of Eugenol is mainly due to its inhibitory effect on prostaglandin synthesis and neutrophils/macrophages chemotaxis (Kim et al., 2003).

5 Conclusion

In conclusion, the Eugenol exhibited renoprotective effects against vitamin D-induced nephrotoxicity. These renoprotective effects could be achieved by the antioxidant and anti-inflammatory activities of the Eugenol. Thus, for vitamin D-induced nephrotoxicity, the use of Eugenol could be beneficial.

Conflict of interest

The authors declare no conflicts of interest.

Availability of data and material

The data used to support the findings of this study are included within the article.

Author contributions

All the authors contributed equally to this work.

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