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Research Article
Protective Effect of *Hypericum perforatum* Extract on Gentamicin Induced Nephrotoxicity in Mice

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Abstract

Background and Objective: *Hypericum perforatum* is a plant which blooms between July and September at farms, borders of roads and woods, top of hills and grasslands, whose anti-inflammatory effects are shown in various studies. Due to this reason, a study is planned to examine the protective effects of *Hypericum perforatum* on nephrotoxicity caused by gentamicin. **Materials and Methods:** Gentamicin is administered to mice for 9 days to form nephrotoxicity and *Hypericum perforatum* extract is administered to mice for 9 days to evaluate protective effects. Cyclooxygenase-2, phospholipase A2 and inducible nitric oxide synthase enzymes in their kidneys are analysed by using ELISA method to compare the nephrotoxicity levels. **Results:** Gentamicin administration increased the expression of cyclooxygenase-2, phospholipase A2 and inducible nitric oxide synthase enzymes. *Hypericum perforatum* extract administration to mice that are administered gentamicin previously decreased the rate of increase of the cyclooxygenase-2 and inducible nitric oxide synthase caused by gentamicin while, it didn't have any effect on phospholipase A2 increase. **Conclusion:** Gentamicin caused an increase in cyclooxygenase-2, phospholipase A2, inducible nitric oxide synthase enzymes in kidneys. Application of *Hypericum perforatum* extract decreased the increase of cyclooxygenase-2 and nitric oxide synthase enzymes significantly while having no effect on the increase of phospholipase A2. In conclusion, this study shows that the gentamicin administration causes nephrotoxicity and the use of *Hypericum perforatum* extract can be helpful against this toxic effect.

Key words: Gentamicin, nephrotoxicity, *Hypericum perforatum*, cyclooxygenase-2, phospholipase A2, inducible nitric oxide synthase

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hypericum perforatum is a plant which blooms between July and September at farms, borders of roads and woods, top of hills and grasslands, that is used to cure some diseases by the local people[1]. This plant contains:

- Flavonoids (Epigallocatechin, rutin, hyperoside, isoquercetin, quercitrin, quercetin, amentoflavone, biapigenin, apigenin, astilbin, myricetin, miquelianin, kaempferol and luteolin)
- Phenolic acids (Chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, p-hydroxybenzoic acid and vanillic acid)
- Naphtodianthrones (Hypericin, pseudohypericin, protohypericin and proto-pseudohypericin)
- Phloroglucinols (Hyperforin and adhyperforin)
- Tannins (Proanthocyanidins)
- Volatile fatty acids (2-methyloctane, nonane, 2-methyldecane, undecane, α-pinene, β-pinene, α-terpineol, geraniol, myrcene, limonene, caryophyllene and humulene)
- Saturated fatty acids (Isovaleric acid (3-methylbutanoic acid), myristic acid, palmitic acid and stearic acid)
- Alkanols (1-tetracosanol and 1-hexacosanol)
- Vitamins and vitamin analogues (Carotenoids, choline, nicotinamide and nicotinic acid)
- Other substances (Pectin, β-sitosterol, hexadecane, triacontane, kielcorin and norathyriol)[2,3]

Anti-inflammatory effects of this plant are shown in various studies. Inhibitory effects of *Hypericum perforatum* on lipopolysaccharide (LPS) induced cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) enzymes are shown in a study[4]. Besides that, hyperforin, which is one of the bioactive compounds of *Hypericum perforatum* is shown to be a strong inhibitor of cyclooxygenase-1 and 5-lipooxygenase[5]. Furthermore, *Hypericum perforatum* application also reduces the prostaglandin synthesis which plays an important role on prostaglandin inflammation[6].

Various studies shown that one of the main reasons for acute intrinsic renal insufficiency is aminoglycoside nephrotoxicity. Nephrotoxicity can be defined as nephrotoxic renal insufficiency. Kidneys are prone to be affected by toxic effects of the drugs and other endogenic and exogenic toxins due to their function in blood perfusion, metabolic activity and excretion. Renal tubular cells are exposed to high toxin concentrations more than other tissues because of active tubular secretion, reabsorption and urine concentration mechanisms in kidneys. Due to this reason, renal tubules are direct targets of nephrotoxicity. Tubular necrosis is the significant feature of the nephrotoxicity. Due to this, it is also called acute tubular necrosis[7].

Aminoglycoside antibiotics are widely used in control and treatment of the Gram (-) aerobic infections. Although gentamicin has the most widespread application areas among these antibiotics, its usage is limited because of its nephrotoxic effects. Aminoglycoside antibiotics are responsible for nearly 10% of the acute renal insufficiency incidences. Due to this, aminoglycoside antibiotics are used as a model in acute renal insufficiency incidences[7].

Various studies showed that the cyclooxygenase pathway, which functions in inflammation, accompanies gentamicin nephrotoxicity. In a study, selective COX-2 inhibitor is reported to be decreasing the nephrotoxicity caused by gentamicin[8]. Furthermore, gentamicin application is determined to be causing an increase in the activity of p-hospholipase A2[9]. In another study conducted, nephrotoxicity formed by gentamicin application caused an increase in iNOS enzyme[10].

The aim of the study is to evaluate the effects *Hypericum perforatum* extract, which is shown to have anti-inflammatory effects, on iNOS, phospholipase A2 and COX-2 enzymes whose levels are increased in nephrons by the gentamicin.

MATERIALS AND METHODS

Male mice (8 weeks old, balb/c, albino) that are obtained from the Experimental Animal Centre in Çukurova University, in Adana are used in study. This study was approved by the Animal Care Committee and Ethics Committee of Cukurova University.

Mice are divided into three groups which are: Control group, gentamicin group and *Hypericum perforatum* extract group. To gentamicin group, 100 mg kg^{-1} gentamicin is applied intraperitoneally once a day for 9 days. To *Hypericum perforatum* extract group, 100 mg kg^{-1} gentamicin is applied intraperitoneally together with 70 mg kg^{-1} *Hypericum perforatum* extract which is applied by using a gavage for 9 days. Physiological serum is applied intraperitoneally to control group under same experimental conditions for 9 days. At the end of the protocol described above, cervical dislocation is applied to the mice. Kidneys of the mice are stored in eppendorf tubes at -80°C for later use in quantitative analysis.

Plant extraction: *Hypericum perforatum* plant is grinded vigorously after being dried in an incubator. Then, it is mixed

with 80% alcohol in 12:1 (Alcohol: Plant) ratio and put in shaker for 24 h at room temperature. After 24 h, it is filtered then alcohol is evaporated by using an evaporator and plant extract is obtained.

Quantitative analysis

Tissue homogenization: Frozen tissue samples that are stored in eppendorf tubes are treated with 3 mL g^{-1} RIPA (Radio-immunoprecipitation assay) buffer, 30 µL PMSF (phenylmethanesulfonyl fluoride), 30 µL sodium vanadate and 30 µL protease inhibitor. Then, homogenates are obtained by using ultra sonication on those tubes on ice. Homogenates are then centrifuged at 10,000 rpm for 10 min and supernatants are taken and pellets are discarded.

Protein quantification: Bradford method is used to quantify the protein in homogenized tissues. By using bovine serum albumin (1 µg mL^{-1}), 1, 2, 3, 5, 7, 8, 10 (µg mL^{-1}) standards are prepared. Then, 10 µL is taken from every sample and completed to 100 µL by adding distilled water. Lastly, 1 mL Bradford solution is added to standards and samples, vortexed and absorbance at 595 nm are measured manually. Protein quantification (µg $µL^{-1}$) is done according to the standard curve drawn in Prism software.

Enzyme linked immunosorbent assay (ELISA) test: The ELISA test is used to examine the expression and activity of COX-2, phospholipase A2 and iNOS enzymes.

Statistical analysis: Results were expressed as Means±SEM and n refers to the number of animals used for each experiments. Differences in results between tissues were tested by analysis of variance (ANOVA) corrected for multiple comparisons (Bonferroni corrections). The p-values less than 0.05 were considered to be significant.

RESULTS

ELISA COX-2 enzyme quantification: While gentamicin application caused an increase in the COX-2 enzyme in kidneys, application of *Hypericum perforatum* extract decreased this increase significantly (Fig. 1). Mean values of COX-2 concentrations for control, gentamicin and gentamicin+*Hypericum perforatum* extract groups are found to be 9499 pg mL^{-1} (SEM 241.2), 11422 pg mL^{-1} (SEM 461.1), 9327 pg mL^{-1} (SEM 298.5), respectively.

ELISA phospholipase A2 enzyme quantification: While gentamicin application caused an increase in the phospholipase A2 enzyme in kidneys, application of *Hypericum perforatum* extract had no effect on this increase (Fig. 2). Mean values of phospholipase A2 concentrations for control, gentamicin and gentamicin+*Hypericum perforatum* extract groups are found to be 1766 pg mL^{-1} (SEM 72.9), 2463 pg mL^{-1} (SEM 115.0), 2639 pg mL^{-1} (SEM 170.6), respectively.

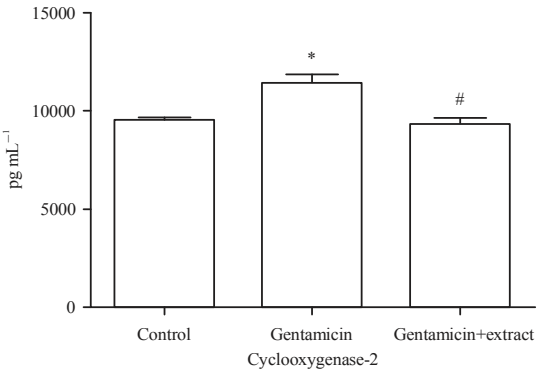

Fig. 1: Effect of *Hypericum perforatum* extract on cyclooxygenase-2 enzyme of the gentamicin applied mice (n = 6). Statistical analysis: ANOVA. *Post hoc*: Bonferroni. *Control p<0.05, #Gentamicin p<0.05

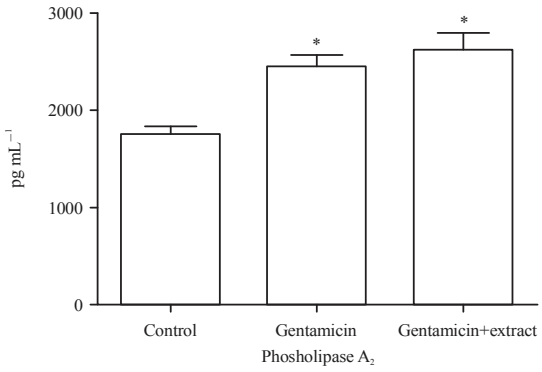

Fig. 2: Effect of *Hypericum perforatum* extract on phospholipase A2 enzyme of the gentamicin applied mice (n = 6). Statistical analysis: ANOVA. *Post hoc*: Bonferroni. *Control p<0.05, #Gentamicin p<0.05)

ELISA iNOS enzyme quantification: While, gentamicin application caused an increase in the iNOS enzyme in kidneys, application of *Hypericum perforatum* extract decreased this increase significantly (Fig. 3). Mean values of iNOS concentrations for control, gentamicin and gentamicin+ *Hypericum perforatum* extract groups are found to be 2251 IU mL^{-1} (SEM 191.1), 3964 IU mL^{-1} (SEM 475.5), 2780 IU mL^{-1} (SEM 108.0), respectively.

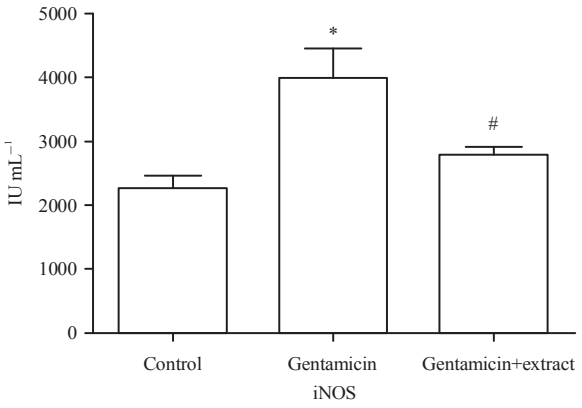

Fig. 3: Effect of *Hypericum perforatum* extract on iNOS enzyme of the gentamicin applied mice (n = 6). Statistical analysis: ANOVA. *Post hoc*: Bonferroni. *Control $p<0.05$, #Gentamicin $p<0.05$)

DISCUSSION

In this study, the enzymes iNOS, phospholipase A2 and COX-2 which functions in inflammatory response as biological mediators is examined. Various studies confirmed the importance of inflammation in nephrotoxicity caused by gentamicin[8-10]. Nitric oxide produced by one of these mediators, iNOS have a pathogenic role in acute and chronic inflammatory diseases[11]. Studies showed that nephron damage caused by gentamicin is accompanied by iNOS enzyme[10]. The increment in LPS induced iNOS enzyme is shown to be decreased by the *Hypericum perforatum* plant that is used in this study[4]. According to the results of this study, application of *Hypericum perforatum* extract also decreased the increment of iNOS enzyme caused by gentamicin.

The COX-2 enzyme, which is an inflammatory mediator and prostaglandins synthesized by this enzyme also plays a role in gentamicin nephrotoxicity and there are studies showing that this enzyme increases in nephrons on gentamicin application[4]. Studies showed that selective COX-2 inhibitors reduce the risk of nephrotoxicity caused by gentamicin. At the same time, *Hypericum perforatum* plant is shown to decrease the LPS induced COX-2 enzyme. Results of this study also shows similarities to previous studies and *Hypericum perforatum* extract decreased the increment caused by gentamicin in COX-2 enzyme.

Another enzyme examined in this study, phospholipase A2 is also shown to be increased by gentamicin application in various studies[9]. According to the results of this study, application of *Hypericum perforatum* extract couldn't decrease this increment. Studies showed that, gentamicin application induces the oxidative stress which causes an increase in intracellular calcium levels and this increase causes an increase in the activity of phospholipase A2 enzyme as a result[12-14]. *Hypericum perforatum* extract have antioxidant properties because of flavonoids and phenolic compounds it contains[15,16]. While researchers expected to see a decrease in increment of phospholipase A2 caused by gentamicin due to application of antioxidant *Hypericum perforatum* extract, it had no effect according to the results. This can be due to the insufficient effect of antioxidant properties of *Hypericum perforatum* extract in nephrons or gentamicin causing this increment via other mechanisms.

This study showed that *Hypericum perforatum* extract decreased the inflammation caused by gentamicin. Pharmacologically active compounds that have anti-inflammatory effects contained by *Hypericum perforatum* are responsible for his effect[15]. Also, antioxidant compounds such as hypericin, flavonoids and hyperforin contained by *Hypericum perforatum* can contribute synergistically to anti-inflammatory effect. Studies showed that hypericin is a free radical scavenger and it inhibits IL-1a, IL-12 formation and arachidonic acid release from phospholipids by affecting 5-lipoxygenase and 12-lipoxygenase pathways. Additionally, NF-kB, which is s regulator of inflammatory mediators, is reported to be inhibited by hypericin[17,18]. Hyperforin, which is another active ingredient is shown to inhibit the formation of free oxygen radicals, elastase release from leukocytes, cyclooxygenase-1, 5-lipooxygenase and IL-6 release[19,20]. Hyperoside and isokuarsitrine, which are flavonoids are shown to inhibit the neutrophil elastase that plays role in inflammation pathogenesis, while hyperoside is inhibiting nitric oxide synthase, isoquercetin is shown to inhibit prostaglandin biosynthesis and release[5,21]. In addition to this, amentoflavone, a flavonoid is shown to inhibit, COX-2, phospholipase A2, iNOS and arachidonic acid release from neutrophils[22-27].

CONCLUSION

In conclusion, this study shows that, application of *Hypericum perforatum* extract reduces the inflammatory response due to increased iNOS and COX-2 enzymes and renal damage caused by gentamicin by inhibiting these enzymes. Thus, application of *Hypericum perforatum* extract reduces the nephrotoxicity risk caused by gentamicin.

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