

THE PROTECTIVE ROLE OF PROPOLIS ON GENTAMICIN-INDUCED NEPHROTOXICITY IN ALBINO RATS

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ABSTRACT

The present work investigate the effect of propolis on gentamicin induced nephrotoxicity in rats. Male Wistar rats were divided into 4 groups; saline, gentamicin (100 mg/kg b.w, i.p., intraperitoneally) for 8 consecutive days, propolis PR (200 mg/kg b.w., p.o.), for 14 consecutive days, propolis 14 days and concurrently with gentamicin for 8 days. Blood urea, serum creatinine, plasma malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) activities and microscopic examination of kidney were performed after the treatment. The results showed that gentamicin treatment caused nephrotoxicity evidenced by marked elevation in blood urea and serum creatinine. Blood urea and serum creatinine were increased in animals treated with gentamicin compared to saline-treated animals. Co-administration of propolis with gentamicin ameliorate blood urea and serum creatinine. GEN administration to rats increased renal MDA and decreased SOD and CAT activities. PR administration with GEN injections significantly decreased MDA, and increased SOD and CAT activities when compared with GEN group.

KEYWORDS: Propolis ; Gentamicin; Reactive Oxygen Species; Renal toxicity.

INTRODUCTION

Neprotoxicity induced by gentamicin (GEN is a complex phenomenon characterized by an increase in blood urea and serum creatinine

concentration, and severe proximal renal tubular necrosis followed by deterioration and renal failure (Al-Majed et al., 2002). Although the pathogenesis is still not well understood, the toxicity of GEN in the kidney seems to relate to the generation of destructive reactive oxygen species (ROS) in these cells (Reiter et al., 2002). ROS have been implicated in a wide range of biological functions, but they can express both beneficial and highly toxic effects on cellular homeostasis (Mates, 2000). A large body of in vivo and in vitro evidence indicates that ROS are important mediators of GEN-induced nephrotoxicity (Kopple et al., 2002). ROS have been proposed as a causative agent of cell death in many different pathological states as well as, in glomerular disease (Smetana et al., 1988), in renal ischemia and reperfusion injury (Longoni et al., 2002), and in various models of toxic renal failure (Piotrowski et al., 1996). Several studies have demonstrated that various agents including melatonin (Ozbek et al., 2000), vitamin E, lipoic acid (Al-Majed et al., 2002), ginkgo biloba extract (Maldonado et al., 2003) can prevent GEN-induced renal damage.

Propolis, a resinous substance that honeybees produce by mixing their own waxes with resins collected from plants, is used as a sealant and sterilizing agent in honeybee nests (Nakajima et al., 2007). Propolis has been used also as a folk medicine in many countries for its particular biological properties in the treatment of cancer and as an antioxidant, anti-microbial, anti-inflammatory and antibiotic agent (Marcucci, 1995 and Banskota et al., 2002). The flavonoids, aromatic acids and phenolic compounds are responsible for the most biological and pharmacological activities of propolis (Vennat et al., 1995). The current work aims to study the effect of propolis against gentamicin-induced nephrotoxicity in albino rats.

MATERIALS AND METHODS

Animals

Forty male albino rats, weighing 200 - 250 g were used in this study; they were housed under conditions of controlled temperature and were

fed standard rat chow. Animals were divided into four groups of 10 animals each. First group includes animals that received only saline throughout the course of the experiment. Animals of the second group received daily intraperitoneally injection of gentamicin (100 mg/ kg/b.w) for eight days, (Abdel-Gayoum et al., 1994). Animals of the third group were given gentamicin (100mg/kg b.w) intraperitoneally for eight days in addition to 200 mg/kg propolis (p.o.) for 14 days. Group four, animals of this group received propolis (200 mg/kg b.w) per orally for 14 days .

At the end of experiment, rats were killed and the kidneys quickly removed, decapsulated and divided equally into two longitudinal sections. One of these was placed in 10% neutral formalin solution for routine histological examination by light microscopy. The other half was placed in liquid nitrogen and stored at -85°C until assayed for MDA, SOD and CAT activities. Trunk blood was extracted to determine the serum levels of blood urea and Creatinine. For these studies, PR (natural product) was dissolved in saline. PR was administered at a dose level of 200mg/kg b.w which was reported that completely blocks production of ROS (Ozyurt et al., 2001).

Biochemical investigations:

Serum Creatinine level was determined using Olympus Autoanalyzer (Olympus Instruments, Tokyo, Japan) and blood urea concentration was determined by GLDH-Kinetic method, using Beck man Spectrophotometer.

Renal antioxidant enzyme activities:

SOD activity determination was based on the production of H_2O_2 from xanthine by xanthine oxidase and reduction of nitroblue tetrazolium as previously described (Goering et al., 2002). The product was evaluated spectrophotometrically at 560 nm. CAT enzyme activity was determined according to Aebi's method (Aebi , 1974). The principle of the assay is based on the determination of the rate constant or the H_2O_2 decomposition rate at 240 nm.

Malondialdehyde (MDA) level:

MDA level in the homogenates were assayed spectrophotometrically at 535 nm according to Mihara and Uchiyama (1978). A standard calibration curve was drawn by using 1,1,3,3-tetramethoxypropane.

Histological observation

For light microscopic evaluation, portions of each kidney were fixed in 10% neutral – formalin, dehydrated in an ascending series of ethanol, cleared in xylene and embedded in paraffin. Tissue sections of 6 μ m were stained with hematoxylin and eosin.

Statistical analysis

Kidney MDA, SOD, CAT, serum urea and Creatinine levels were analyzed by one-way ANOVA. Posthoc comparisons were done using Tukey's tests. Differences were considered significant at $P < 0.05$. Results are expressed as mean \pm SD.

RESULTS

1-Effect of PR on GEN-induced changes in serum parameters.

Table 1 showed that serum levels of blood urea and creatinine in the GEN-treated animals are significantly higher than control group [27.4 \pm 2.3 versus 58 \pm 3.7; 0.35 \pm 0.02 versus 0.76 \pm 0.02, respectively].

Sera of animals treated with both GEN and PR showed significantly reduced urea and creatinine levels when compared with animals of GEN group [58 \pm 3.7 versus 32.6 \pm 4.1; 0.76 \pm 0.02 versus 0.44 \pm 0.03, respectively]. There were no statistically differences between control and PR treated groups.

2- Effect of PR on GEN-induced changes in kidney tissue enzymes, lipid peroxides.

Data in Table 1. GEN-induced acute renal failure manifested by a significantly increased kidney MDA level [61.44 ±7.40versus 100.79±2.56] and significantly decreased SOD,CAT activities [158.1±4.49 versus 137.71 + 2.41 and 5.83±0.28versus 1.74±0.31, respectively]. PR administration with GEN injections caused significant decrease in MDA[100.79±2.56versus 71 + 4.83] and increased in SOD, CAT activities [164.36±7.90versus 164.36 ±7.90; and 1.74±0.31versus 4.02±0.23; respectively] in kidney when compared with GEN. There were no statistical differences between control and PR-treated groups.

Table 1: Effects of gentamicin (GEN) administration on rats with or without propolis (PR) on blood urea and creatinine levels,kidney MDA level and SOD&CAT activities.

Parameters	Control	GEN	GEN+ PR	PR
Blood urea (mg/dL)	27.4 ±2.3	58 ±3.7 ^(a)	32.6 ±4.1 ^(b)	27.7 ±1.9
Creatinine (mg/dL)	0.35±0.02	0.76 ±0.02 ^(a)	0.44 ±0.03 ^(b)	0.37±0.03
MDA (nmol/gtissue)	61.44 ±7.4	100.79±2.56 ^(a)	71 ± 4.83 ^(b)	66.32±3.0
SOD (U/gm tissue)	158.1±4.49	137.71±2.41 ^(a)	164.36±7.90 ^(b)	154.45±5.61
CAT (k unit/ml)	5.83±0.28	1.74±0.31 ^(a)	4.02±0.23 ^(b)	5.98±0.34

Data are shown as means ± SD

^(a) P < 0.05 versus control group.

^(b) P < 0.05 versus GEN group.

3- Effect of PR on GEN-induced morphological changes in kidney tissue:

The histological results are showed some morphological changes in kidneys. Kidneys of animals of control group showed normal kidney structure (Fig. 1a). On the other hand, there were not any microscopical differences between the control and only PR-treated groups. GEN-treated rats. More extensive and marked tubular necrosis was seen (Fig. 1b). In

the GEN+ PR-treated rats, sparse tubular changes were observed (Fig. 1c). PR apparently reduced kidney tissue damage.

DISCUSSION

The nephrotoxicity of aminoglycoside antibiotics, and specially that of the most commonly used compound, gentamicin, is well documented (Cuzzocrea et al., 2002; Al-Majed et al., 2002). Several studies have reported that oxygen-free radicals are considered to be important mediators of GEN-induced acute renal failure (Karahan et al., 2005). Accordingly, among the main approaches used to ameliorate GEN - induced nephrotoxicity is the use of agents with powerful antioxidant properties. Several recent studies have reported that the propolis or its components may be useful in ameliorating signs of GEN nephrotoxicity (Parlakpınar et al., 2005). In this study, the antioxidant properties of PR to prevented the nephrotoxicity by improving histopathological changes. Several dosages have been reported for GEN administration. In the present study, acute nephrotoxicity was created by injecting GEN (100 mg/kg b.w i.p.). In the present study, PR is a potent antioxidant and free-radical scavenger on the renal damage and oxidative injury induced by GEN.

Plasma creatinine concentration is a more potent indicator than the urea concentration in the first phases of kidney disease. Furthermore, urea concentration begins to increase only after parenchyma tissue injury (Gilbert et al., 1989). In this study plasma creatinine and urea levels were higher ($p < 0.05$) in the GEN group when compared with the control group. So, the elevation in blood urea & creatinine levels in GEN treated rats is considered as marker of renal dysfunction. This result is in agreement that reported by Kopple et al. (2002), Parlakpınar et al. (2005)

In the current study, GEN induced oxidative stress which results in lipid peroxidation causing increase in MDA levels and decrease in antioxidant enzymes like catalase and superoxide dismutase CAT is a hemoprotein which catalyses the reduction of hydrogen peroxide and

protects the tissues from highly reactive hydroxyl radical (Rajasekaran et al., 2005). The reduction in the activities of this enzyme could reflect the adverse effect of GEN. Furthermore, the propolis treatment prevented depletion of CAT activity induced by gentamicin. The protective effect might be due to the ability of propolis to inhibit hydrogen peroxide-induced oxidative injury in renal cell line (Parlakpınar et al., 2005).

In the current study, GM induced oxidative stress which results in decrease in antioxidant enzymes like catalase and superoxide dismutase (SOD). There are some experimental data suggesting that nephrotoxic drugs may also change levels of MDA, glutathione peroxidase (GSHPx), CAT, SOD, GSH, BUN and Cr (Ozbek et al., 2000) which are commonly used to monitor the development and extent of renal tubular damage due to oxidative stress.

Thus, the preventive effect of propolis on the gentamicin induced decrease in the activity of superoxide dismutase (SOD) and CAT could be contributed to the restoration of markers of renal tubular injury. It seems reasonable to assume that propolis is able to suppress nephrotoxicity in kidney as it was demonstrated in studies with gentamicin (Parlakpınar et al., 2005) and (Vardi et al., 2005), amikacine (Parlakpınar et al., 2005) and doxorubicin (Yagmurca et al., 2005).

The effects of CAPE on GEN-induced renal changes using both biochemical determinations and the morphology of the kidney using light microscopy (Parlakpınar et al., 2005).

In this study, it has been shown that GEN, at 100 mg/kg, significantly increased the level of lipid peroxidation products (MDA) suggesting the involvement of oxidative stress.

On the other hand, the effect of GEN and elevation of the lipid peroxidation product, MDA, were reduced by propolis treatment which are in line with various previous reports. The present results showed that propolis decreases lipid peroxidation possibly by its antioxidant activity.

Parlakpınar et al., (2005) reported a protective effect of propolis on circulating lipids in plasma and on lipid peroxidation products in alcohol and on polyunsaturated fatty acid induced toxicity. Lipid peroxidation, mediated by oxygen-free radicals, is believed to be an important cause of destruction and damage to cell membrane. Malondialdehyde is formed during oxidative degeneration and is accepted as an indicator of vancomycin-induced injury and that supplementation with CAPE may be helpful in reducing vancomycin nephrotoxicity (Iwamoto et al., 2003).

Histopathological results demonstrating structural changes in renal tissue of aminoglycoside antibiotics such as GEN were reported by some researchers (Kumar et al., 2000; Al-Majed et al., 2002; Ateşşahin et al., 2003; Polat et al., 2006; Sayed-Ahmed and Nagi, 2007). The histopathological results in this study are in rapport with these reported studies. In the present study, Glomerular and tubular epithelial changes were considerably mild in the groups treated with PR. The histopathological results in connection with protective effects of PR or its component for induced nephrotoxicity of aminoglycosides including GEN were in agreement with other reports (Parlakpınar et al., 2005). We think that, morphological changes in kidneys were because of GEN injection, but these changes tended to be considerably mild in GEN plus PR injection.

In conclusion, the GEN -induced nephrotoxicity may be related with oxidative damage. Co-administration of PR decreased the harmful effects of GEN both by inhibiting free-radical formation and by restoration of the antioxidant systems. Further investigations on the mechanism of action of PR are required and may have a considerable impact on future clinical treatments of patients with renal failure.

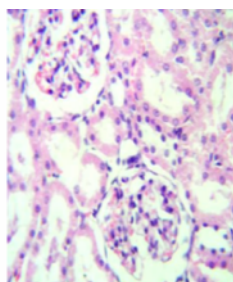


Figure 1a. Tubules appear normal in the control group. H-E X 66.

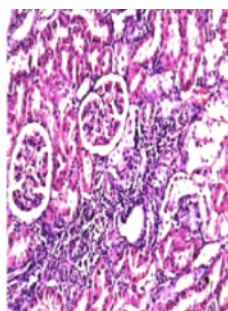


Figure 1b. tubular necrosis is observed in the GEN-treated group. H-E X 66

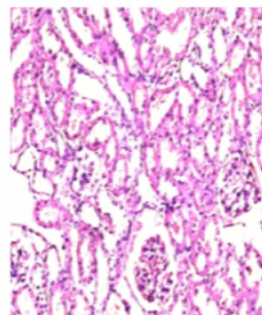


Figure 1c. Tubules show slight histological changes in the GEN+ PR -treated group .The tubules revealed normal. H-E X 66.

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الدور الوقائي لصمغ النحل(البروبوليس) على التسمم الكلوي المحدث بالجنتاميسين فى الجرذان البيضاء.

أبراهيم حلمى ، طارق سالم ، أيمن المغاوري ، خالد بسيونى، ماجدولين قابل

قسم البيولوجية الجزيئية-معهد الهندسة الوراثية والتكنولوجيا الحيوية-جامعة المنوفية.

الهدف من البحث تحديد تأثير صمغ النحل(البروبوليس)على التسمم الكلوي المحدث بالجنتاميسين فى ذكور الجرذان البيضاء. اشتملت الدراسة على عدد اثنان و ثلاثون (٣٢) فأراً أبيض قسمت إلى خمس (٤) مجموعات كل منها يتكون من ثمانية (٨) فئران. المجموعة الأولى: تتعاشى الجرذان بهذه المجموعة تحت الظروف العادية من حيث التغذية والشراب المجموعة الثانية: تم حقن الجرذان فى تجويف البطن بالجنتاميسين بجرعة مقدارها ١٠٠ ملليجرام/ كليوجرام يومياً لمدة ثمانية أيام متتالية المجموعة الثالثة: تم إعطاء معلق صمغ النحل(البروبوليس)عن طريق الفم بجرعة ٢٠٠ ملليجرام/كليوجرام يومياً لمدة ١٤ يوم متتالية ثم حقن الجرذان فى تجويف البطن بالجنتاميسين بجرعة مقدارها ١٠٠ ملليجرام/ كليوجرام يومياً لمدة ٨ أيام متتالية. المجموعة الرابعة: تم إعطاء معلق صمغ النحل(البروبوليس)عن طريق الفم بجرعة مقدارها ٢٠٠ ملليجرام/كليوجرام يومياً لمدة ١٤ يوم متتالية. وقد خضعت الفئران فى كافة المجموعات لما يلي: تم أخذ عينات الدم من كل فأر وذلك لقياس:مستوي اليوريا فى الدم ومستوي الكرياتين فى الدم. تم ذبح الجرذان والحصول على الكلي والكبد وذلك لقياس مستوي الإنزيمات المضادة للأكسدة و دراسة هستوباثولوجية لوحظ ارتفاع ذي دلالة إحصائية فى مستوي اليوريا والكرياتين ومستوي المالوندايالدهايد وأيضاً إنزيمات الكبد المعاملة بالجنتاميسين بمفردها إذا ما قورنت بالمجموعة الضابطة وأيضاً لوحظ انخفاض فى مستوي الكتاليزو الوسبر او كسيد داى ميوتاز و حدوث تغيرات هستولوجية مرضية فى المجموعة الثانية إذا ما قورنت بالمجموعة الأولى. انخفاض مستوي انزيمات الكبد بنسبة تتقارب من المجموعة الضابطة. ارتفاع مستوي مضادات الأكسدة فى الدم ثبت أن معاملة الجرذان بالجنتاميسين بعد معاملةها بصمغ النحل(البروبوليس) Post treatment فى المجموعة الثالث (PR +GM) تعطي أفضل إمكانية للوقاية من تأثير الجنتاميسين ووضوح الدور الوقائي لصمغ النحل(البروبوليس) .