Potential role of Saudi red propolis in alleviating lung damage induced by methicillin resistant *Staphylococcus aureus* virulence in rats

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Abstract: The aim of this study was to explore the protective impact of aqueous extract of Saudi red propolis against rat lung damage induced by the pathogenic bacteria namely methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 6538 strain. Infected rats were received a single intraperitoneal (i.p.) injection of bacterial suspension at a dose of 1 X 10^6 CFU / 100g body weight. Results showed that oral administration of an aqueous extract of propolis (50mg/100g body weight) daily for two weeks to infected rats simultaneously with bacterial infection, effectively ameliorated the alteration of oxidative stress biomarker, malondialdehyde (MDA), as well as the antioxidant markers, glutathione peroxidase (GPx) and superoxide dismutase (SOD), in lungs of infected rats compared with infected untreated ones. Also, the used propolis extract successfully modulated the alterations in proinflammatory mediators, tumor necrosis factor-α (TNF- α) and vascular endothelial growth factor (VEGF) in serum. In addition, the propolis extract successfully modulated the oxidative DNA damage and the apoptosis biomarker, caspase 3, in lungs of *S aureus* infected rats compared with infected untreated animals. The biochemical results were supported by histo-pathological observation of lung tissues. In conclusion, The beneficial prophylactic role of the aqueous extract of Saudi red propolis against lung damage induced by methicillin resistant *S aureus* may be related to the antioxidant, anti-inflammatory, immunomodulatory and antiapoptosis of its active constituents.

Keywords: Saudi red propolis, methicillin resistant, *Staphylococcus aureus*, oxidative stress, vascular endothelial growth factor

INTRODUCTION

Staphylococcus aureus is anaerobic opportunistic gram-positive bacteria, capable of causing pathology in many tissues of the host. Infection with this pathogen causes a range of diseases including, bacteremia, food poisoning, sepsis, brain abscess, toxic shock syndrome, osteomyelitis, endocarditis and pneumonia (Lowy, 1998). Infection by *S. aureus*, particularly those caused by methicillin-resistant *S. aureus* (MRSA) strains, has been a leading cause of morbidity and mortality (Klevens *et al*., 2007). Pneumonia is the one of the most prevalent disease caused by methicillin-resistant *S. aureus* (MRSA), and the incidence of severe pneumonia caused by MRSA strains is rising (Klevens *et al.,* 2007).

One of the adverse effects of *S. aureus* pneumonia is the intense inflammatory immune response of the host with excessive recruitment of neutrophils. The severe pneumonia caused by this pathogen, due to the intense inflammatory reactions, can lead to the development of lung injury. (Rastogi *et al.,* 2001; Gomez *et al.,* 2004). Previous animal studies have shown that α -toxin, (Bubeck Wardenburg *et al.,* 2007), Panton-Valentine leukocidin (Labandeira-Rey *et al.,* 2007) and protein A (Gomez *et al.,* 2004) are the risk factors of *S. aureus* pneumonia. Some authors reported that these factors are pore-forming toxins and can induce lung inflammatory response and damage by stimulating the over-expression of inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and chemokines and inducing inflammatory cells to release additional inflammatory mediators suggesting that these toxins have the principle role in inducing lung damage (Dragneva *et al.,* 2001; Gomez *et al.,* 2004; Ratner *et al.,* 2006; Cheng *et al.,* 2009). Beside, previous studies revealed that tissue damage caused by *S. aureus* infection attributed to the ability of this pathogen to promote the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) with a concomitant decrease in antioxidant defense system (Kengatharan *et al.,* 1996; Prasad *et al.,* 2011). Imbalance between oxygen reactive species production and antioxidants, is considered one of the risk factors associated with DNA oxidative damage and tissue apoptosis (Simon *et al.,* 2000; Prasad *et al.,* 2011).

Illustrating the mechanisms by which *S. aureus* induces pathogenesis in animal models may clarify its deleterious impact in human infections and provide a strategy for the establishment of new therapeutic interventions. Due to the increasing rate of antibiotic resistances of most bacteria, using of traditional natural agents may be effective as alternative medicine for the treatment of bacterial infection particularly for antibiotic resistant strains.

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Propolis is a resinous mixture, collected from plant buds and exudates by honeybees; this substance is used as a building insulating material in beehives (Greenaway *et al.,* 1990). Propolis has been used widely in the traditional medicine in the west and the east for several years. It has been shown that propolis has many therapeutic properties, such as anti-inflammatory (Paulino *et al.,* 2008), antibacterial (Kujumgiev *et al.,* 1999; Vardar-Unlu *et al.,* 2008; Bastos *et al.,* 2008), antioxidant (Xi and Shouqin, 2007), hepatoprotective (De Barros *et al.,* 2007), and antitumoral (Orsolic and Basic, 2005) activities. Propolis has many chemical constituents, which depend on the local flora at the site of its collection (Marcucci, 1995).

The diversity in the chemical composition of propolis, is attributed to the wide varieties of plant species used by honeybees as sources of resin for the production of propolis. More than 300 compounds have been identified (Lustosa *et al.,* 2008), including terpenoids, lignans, prenylated organic acid derivatives, phenolic compounds, amino acids, fatty acids, vitamins and minerals (Cuesta-Rubio *et al.,* 2007; Duran *et al*. 2008; Salomão *et al.,* 2008). A previous study reported that phenolic acids, phenolic acid esters and flavonoids are the major constituents of the propolis (Bankova, 2005). The therapeutic activities of propolis are attributed to the large variety of compounds of plant origin, which act individually and/or synergistically (Righi *et al.,* 2013).

The current investigation is aimed to explore the possible beneficial role of Saudi red propolis aqueous extract against oxidative tissue damage, immuno-inflammation, oxidative DNA damage and apoptosis induced lung damage in rats in response to infection with methicillin resistant *S aureus* (MRSA) ATCC 6538 strain.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid, 5,5′- dithiobis(2-nitrobenzoic acid), , reduced glutathione NADPH, NADH, EDTA, 2-mercaptoethanol and other chemicals used in the study were of high analytical grade, products of the Sigma and Merck companies.

Propolis sample

Propolis was obtained from a honey bee market, located in Jeddah, Kingdom Saudi Arabia. The whole sample of propolis (200g) was frozen at -18◦C, ground and homogenized to a fine powder prior the extraction

Preparation of the aqueous extract of red propolis

200g of powdered propolis, were added to 500mL of distilled water. The suspension was stirred at 70◦C for 30-60 min and then cooled at the room temperature. The extract was filtered using filter paper (Whatman 1) to obtain the first extract. The residual precipitate was extracted again according to the previous procedure to obtain, the second extract. The first and the second extracts were pooled and then lyophilized (Preethi *et al.,* 2010).

Bacterial strain

The phathogenic bacteria, methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 6538 strain were used in this study. *S. aureus* was collected from the Department of microbiology (National Research Centre, Cairo, Egypt). The confirmatory tests, for the phenotypic identification of the tested bacterial strain, were carried out according to standard protocols by using biochemical and gram staining methods. Following the confirmatory tests, the culture of tested bacterial strain was grown in tryptic broth and incubated for 24 hours at 37˚C, then centrifuged at 15,000 x g for 15 minutes and the pellet was re-suspended in a phosphate buffer saline (PBS). The absorbance was followed at 620 nm, using a UV spectrophotometer (Schimadzu, USA) and the count of the surviving bacterial was adjusted to approximately 1.0 X 10^6 colony forming units (CFU)/ml, (Bouknight and Sadoff, 1975).

Animals and experimental design

Animal experiment was performed with compliance of the local ethics committee. Forty Wistar male albino rats (150-170 g.) were supplied by the Experimental Animal Center, King Abdulaziz University. The animals have been kept in special cages and maintained on a constant 12-h light/12-h dark cycle in an air-conditioned animal house, at 20±2°C and 50-70% relative humidity. The animals were fed a commercial rat pellet diet and tap water *ad libitum*. Animal utilization protocols were performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the College of Science, King Abdulaziz University. After one-week acclimatization, rats were divided into four groups, each of 10 animals as follow

Group 1: Normal healthy rats.

Group 2: Rats orally received aqueous extract of propolis (50mg/100g, Lima deMoura *et al.,* 2009) daily for two weeks.

Group 3: Rats injected intraperitoneally (i.p.) with a single dose (1X 10^6 CFU / 100g body weight) of bacterial suspension

Group 4: Rats infected with bacterial suspension (1X 10^6 CFU / 100g body weight, i.p.) and co-administered orally with aqueous extract of propolis (50mg/100g body weight) daily for two weeks.

Two weeks later, the rats of all experimental groups were fasted overnight (12-14 hours). The blood samples were gathered from each animal in sterilized test tubes and then centrifuged at 4,000 rpm for 15 minutes for serum separation and used for biochemical serum analysis. After blood collection, the animals were sacrificed under ether anesthesia and their lungs were removed and washed with an ice cold saline solution. The lungs were cut and homogenized in a chilled bi-distilled water to yield 10% homogenates, using a glass homogenizer. The homogenate samples were centrifuged for 20 minutes at 5,000 rpm and 4°C. The supernatants were used for the biochemical lung tissue assays.

Biochemical serum assay

The level of inflammatory cytokine such as tumor necrosis factor (TNF-α) in serum was estimated using ELISA assay method following the instructions supplied by the manufacturer (DuoSet kits; R&D Systems, Minneapolis, MN, USA). The concentration of the cytokine is shown as pg/ml. The level of vascular endothelial growth factor (VEGF) was estimated quantitatively at 492 nm using a colorimetric sandwich enzyme-linked immunosorbent assay (ELISA; R&D Systems, UK), according to the instructions of manufacturer. VEGF level was estimated using a standard curve generated by specific standards supplied by the manufacturer.

Biochemical assay of lung tissue

Lipid per oxidation was measured by estimating the produced MDA using thiobarbituric acid reactive substances (TBARS) method (Buege and Aust, 1978). This method is based on the production of red adduct between thiobarbituric acid and MDA in acidic medium. MDA level was evaluated using the extinction coefficient value (ε) of MDA-thiobarbituric acid complex (1.56×10^5 /M/cm). GPx activity was estimated using dithiobis (2-nitrobenzoic acid) assay method (Rotruck *et al*., 1973), depending on the reaction between 5,5′- dithiobis (2-nitrobenzoic acid) and the residue of glutathione after the action of GPx to ithiobis (2-nitproduce a complex. The formed complex can be measured at 412 nm. SOD activity was determined by monitoring the decrease in absorbance of NADH at 340 nm (Paoletti *et al*., 1986). The activity is expressed in terms of % inhibition of NADH.

Comet assay

The single cell gel electrophoresis (comet assay), is a technique for evaluating DNA fragmentation in individual cells. The method used in this study is based on the unwinding of DNA under alkaline media (Singh *et al.,* 1988). In DNA analysis, about fifty cells per sample were analyzed. To analyze the electrophoretic DNA patterns, tail length, tail DNA% (the relative tail DNA content) and the tail moment were estimated. The length of tail was determined from the middle of the head to the end of the tail. The tail moment was estimated by the DNA% in the tail multiplied by the length between the center of the head and tail (Olive *et al.,* 1990).

Caspase 3 assay

Caspase 3-like protease was determined using the method of Vaculova and Zhivotovsky (2008).

Histopathological examination

Lungs were cut into small pieces and fixed using 4% formalin . The lung samples were then embedded into a paraffin, sectioned for 3-4-μm thick and mounted on the glass microscope slides, using standard histopathological techniques. The sections were stained with hematoxylin-eosin and were examined using a light microscope.

STATISTICAL ANALYSIS

The experimental results were statistically analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's test as post-ANOVA. The data were expressed as mean ± SD. Differences between the results of different experimental groups were considered significant at p value of less than 0.05.

RESULTS

The level of the oxidative tissue damage biomarker, MDA, as an index of membrane lipid per oxidation and the antioxidant enzymes, SOD and GPx, in rat lungs of different animal experimental groups are depicted in fig. 1. The result showed that infection of rats with methicillin resistant *S aureus,* ATCC 6538 strain, caused a significant increase in the level of MDA with concomitant decreases in the antioxidant enzymes, SOD and GPx, in lungs of infected rats (G3), compared with the normal group (G1) ($P \leq 0.001$). Oral administration of aqueous extract of Saudi red propolis to infected rats simultaneously with infection, markedly ameliorated the alteration in the oxidative stress and antioxidant biomarkers in infected treated animals (G4) with respect to infected untreated ones ($P \leq 0.001$).

Fig. 2 revealed that methicillin resistant *S aureus* bacterial infection induced elevation in the levels of serum pro-inflammatory cytokine, TNF-α and the angiogenic factor, VEGF, in *S aureus* infected rats versus normal ones ($P \leq 0.001$). Co-ingestion of red propolis aqueous extract to infected rats, significantly reduced the elevated levels of TNF-α and VEGF in infected treated rats in relation to infected untreated animals ($P \leq 0.001$).

The effect of methicillin resistant *S aureus* infection on the DNA of rat lungs is shown in figs. 3 and 4. Significant increases in the tail length, tail DNA% (tail DNA content) and tail moment were shown in the lung tissues of infected rats in relation to normal ones. Oral co-administration of the propolis extract to infected rats, significantly protected their lung tissues from DNA damage as indicated by the decrease in tail length, DNA% and tail moment compared with infected untreated rats.

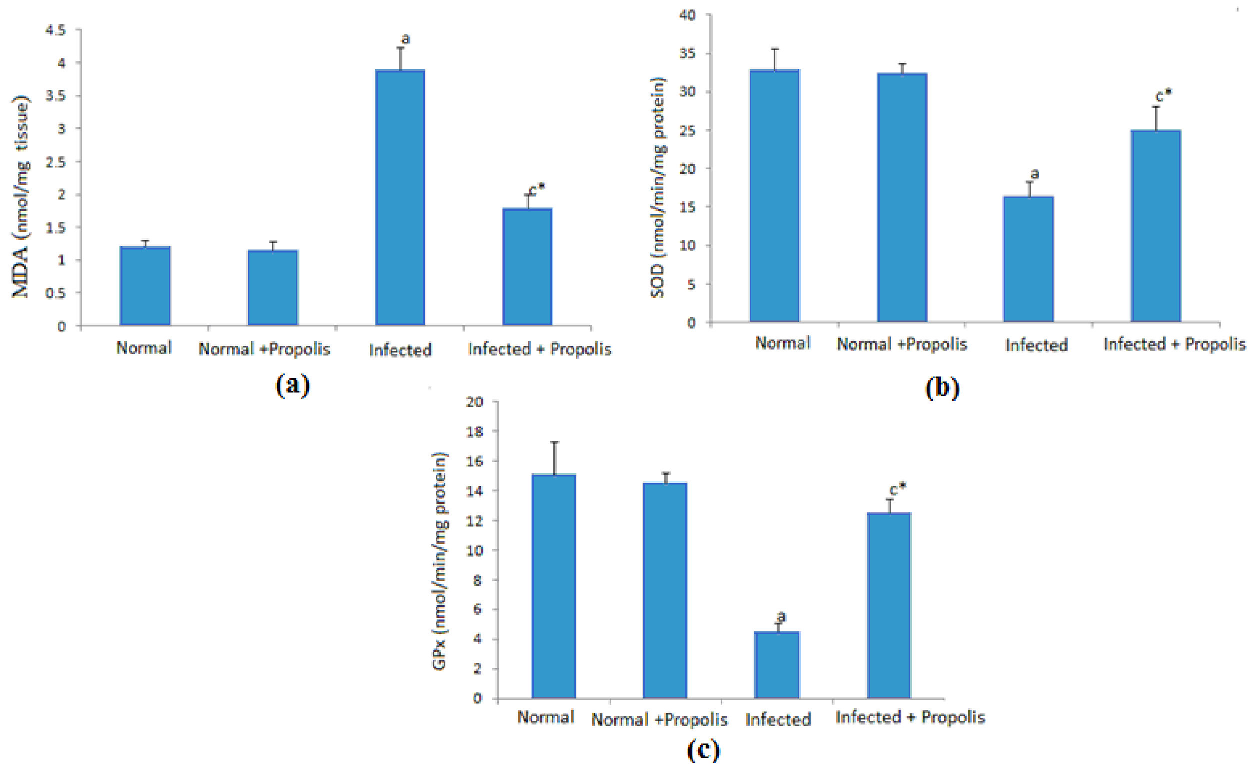

Fig. 1: Levels of oxidative stress and antioxidant biomarkers in lungs of different experimental groups, (a) MDA (index of lipid peroidation), (b) SOD, (C) GPx. Data are presented as mean□±□S.D. of 10 rats, $^{a}P\leq0.001$, $^{c}P\leq0.05$ compared with the normal group, $^{*}P\leq0.001$, compared with infected group, using ANOVA followed by Bonferroni as a post-ANOVA test.

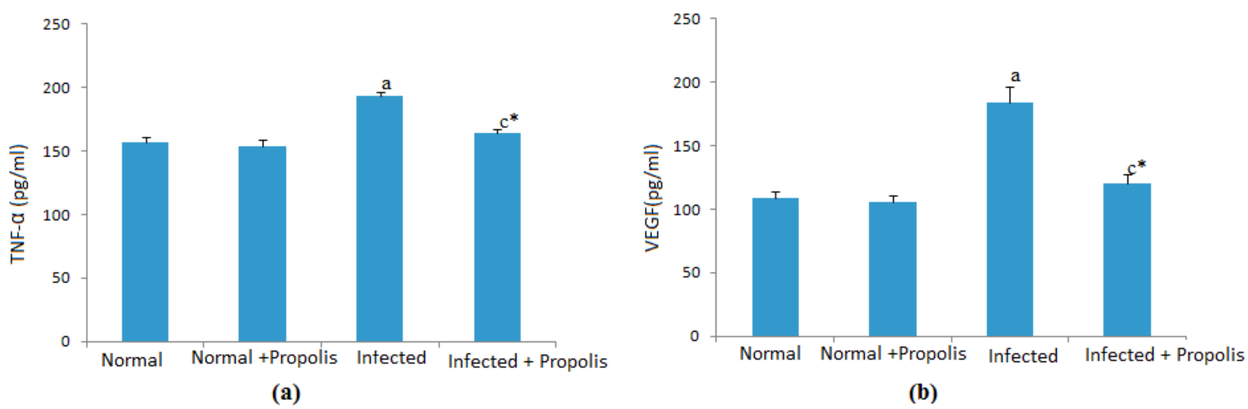

Fig. 2: Levels of proinflammatory cytokine (a) and angiogenic biomarker (b) in sera of different experimental groups. Data are presented as mean±S.D. of 10 rats, $^{a}P\leq0.001$, $^{c}P\leq0.05$ compared with the normal group, $^{*}P\leq0.001$, compared with infected group, using ANOVA followed by Bonferroni as a post-ANOVA test.

Fig. 5 shows that the apoptosis biomarker, caspase 3, was significantly up-regulated in the lungs of *S aureus* infected rats compared with normal animals. Co-administration of the studied propolis extract to infected rats, beneficially down-modulated the increase in the apoptosis biomarker in the lung of infected rats.

The protective role of propolis aqueous extract against histomorphological lung damage induced by *S aureus* infection, is shown in fig. 6. Histopathologic examination showed the consequences of *S. aureus* infection for lung parenchyma. Control lungs of uninfected animals showed normal alveolar architecture, in which thin-walled air spaces were defined by a single layer of pneumocytes and the bronchioles were lined by ciliated epithelium (fig. 6A). Infection of animals with *S. aureus* showed a significant alveolar destruction as observed by thickening of alveolar walls and infiltration of inflammatory immune cells with edema (fig. 6C). Co-administration of propolis aqueous extract to infected rats showed more or less normal histological structure of lung (fig. 6D).

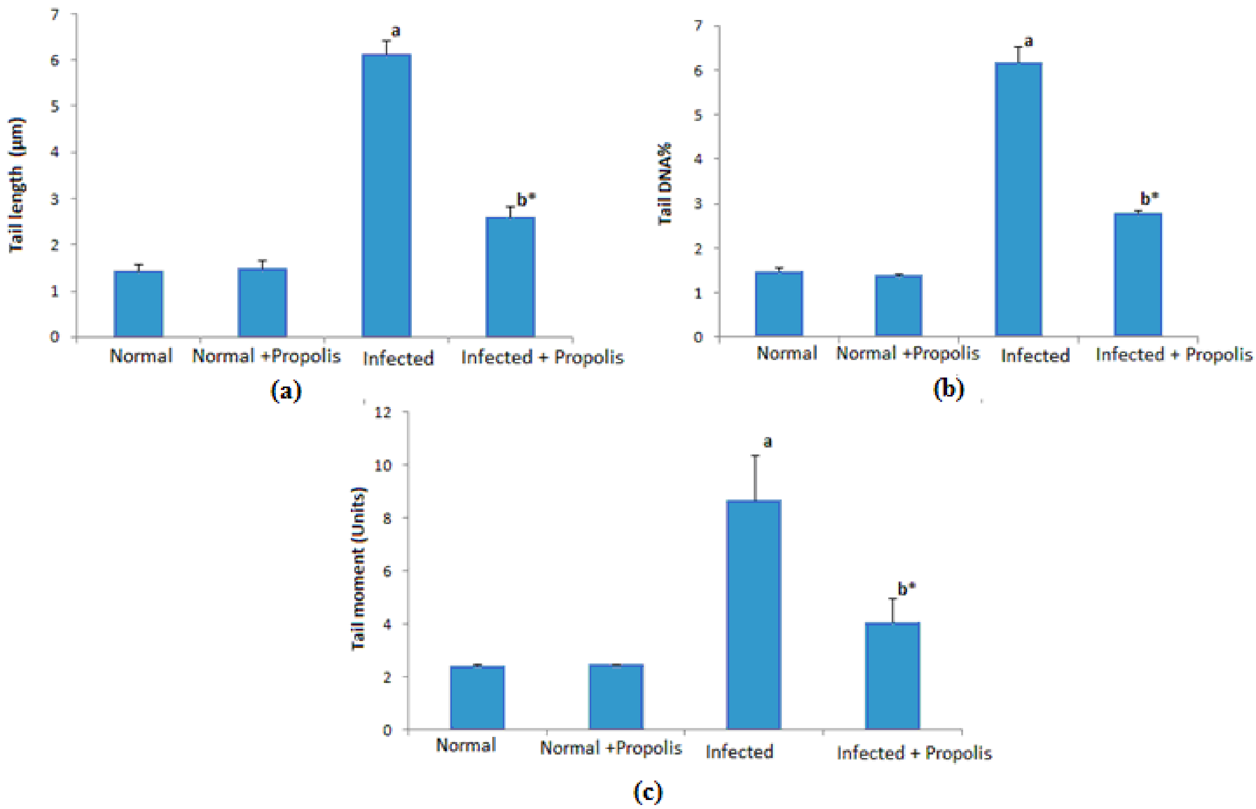

Fig. 3: Levels of DNA damage markers in lungs of different experimental groups, (a) tail length, (b) tail DNA%, (c) tail moment. Data are presented as mean ± S.D. of 10 rats, $^{a}P\leq0.001$, $^{b}P\leq0.01$, compared with the normal group, $^{*}P\leq0.001$, compared with infected group, using ANOVA followed by Bonferroni as a post-ANOVA test.

No significant changes were seen in the studied biochemical parameters (G2) or in the lung histomorphological structure (fig 6B) on treating the normal rats with the used propolis extract compared with normal untreated group.

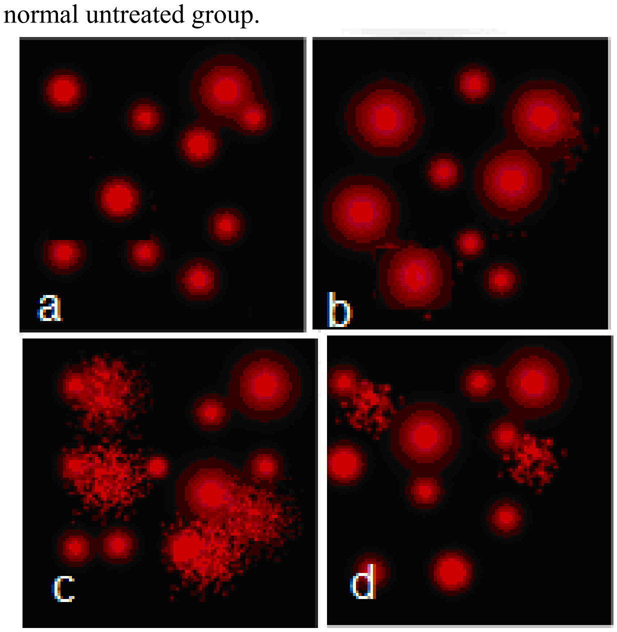

Fig. 4: DNA damage in the lung tissue of *S aureus* infected rats and the effect of propolis extract treatment on the level of DNA damage. COMET assay showing degree of DNA damage in lung tissues of (a) normal control group, (b) normal group ingested propolis extract, (c) animal group infected with *S aureus* and (d) infected group treated with propolis extract.

DISCUSSION

It is well known that the number of bacteria resistant to antibiotic treatments has increased. Bacterial antibiotic resistance has been identified by WHO as one of the three paramount threats to human health. It was reported that several pathogens are becoming resistant to antibiotics, such as Gram positive and Gram negative bacteria (Liu and Pop, 2009). So development of new agents to counter these resistant pathogens become urgent.

In the current study, the beneficial prophylactic effect of red propolis aqueous extract from Saudi Arabia as an alternative medicine against rat lungs damage induced by infection with methicillin resistant *S aureus* (MRSA), ATCC 6538 strain, was investigated. It is well established that bacterial infection -related to methicillin-resistant *S aureus* has been concomitant with a serious oxidative tissue damage and inflammation with a high rate of mortality (Klevens *et al.*, 2007; Cheng *et al.*, 2009; Prasad *et al.*, 2011).

The result of the current study revealed that infection of rats with methicillin-resistant *S aureus* induced oxidative stress in the lungs of infected rats as shown by an increase in MDA level as an index of membrane lipid per oxidation with concomitant decreases in the antioxidant marker enzymes (SOD and GPx) in lungs of infected rats compared with normal healthy ones. Similar results were obtained in livers and kidneys of animals in response to infection with this pathogen (Chakraborty *et al.*, 2011; Prasad *et al.*, 2011). The increase in lipid per oxidation and the decreases in antioxidant enzymes induced in lungs of *S aureus* infected rats can be used as *in vivo* indicators of over production of oxygen-free radical (Natarajan *et al.*, 2007). The imbalance between ROS production and the antioxidant defense system can be used as a marker for the oxidative stress and the tissue injury. Lipid per oxidation can lead to an increase in the rate of protein degradation, changes in cell membrane permeability and fluidity, causing eventually to cell lysis. It is well known that antioxidant defense system such as SOD and GPx enzymes, can protect tissues from the deleterious impacts of reactive species (Olga Blokhina *et al.*, 2003) . SOD, is the first one in the antioxidant enzymatic defense system, plays a fundamental role in the dismutation of super oxide leading to hydrogen peroxide formation which is then neutralized by catalase and GPx (Olga Blokhina *et al.*, 2003). The reduction in SOD and GPx activities leads to assemblage of super oxide and hydrogen peroxide ions, which can lead to an increase in the product of lipid per oxidation in the lungs of *S. aureus* infected rats.

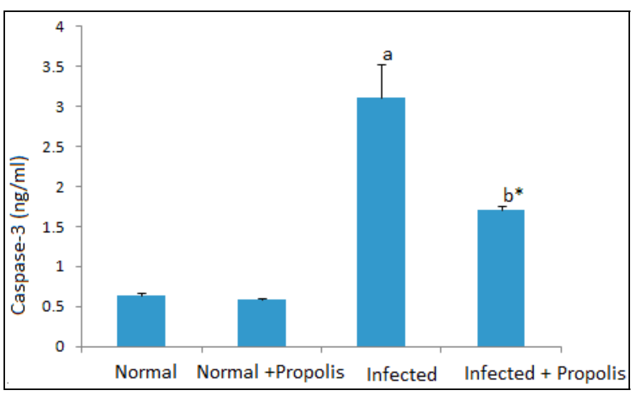

Fig. 5: Level of apoptosis biomarker, caspase 3, in lungs of different experimental groups. Data are presented as mean ± S.D. of 10 rats, $^{a}P\leq0.001$, $^{b}P\leq0.01$, compared with the normal group, $^{*}P\leq0.001$, compared with infected group, using ANOVA followed by Bonferroni as a post-ANOVA test.

Oral administration of propolis aqueous extract to infected rats simultaneously with infection, succeeded to ameliorate the lung damage induced in rats by methicillin-resistant *S aureus* infection as evidenced by the reduction in the level of lipid peroxide and the elevation in antioxidant marker enzymes (SOD and GPx) in lungs of infected treated rats in relation to infected untreated animals. This result may indicate that the aqueous extract of the used propolis contains active compounds with a potential antibacterial action and/ or a high anti-oxidant effect. The antimicrobial activities of Saudi propolis toward different drug multi-resistant pathogens including *S aureus*, in a single and polymicrobial cultures, were previously documented (AL-Waili *et al.*, 2012). Beside, chemical analysis of Saudi propolis has revealed the predominant of phenolic compounds (caffeic, *p*-coumaric, *trans*- cinnamic, sinapic and ferulic) and flavonoids (kaempferol, apigenin, rutin, quercetin) which exhibit antimicrobial and antioxidant potential effects (Abd El-Mawla and Osman, 2011). Also, a previous study showed that aqueous extract of propolis could inhibite the oxidative stress induced by lipid peroxidation in livers of experimental animal models (El-Khatib *et al.*, 2002). Furthermore, propolis was reported to have the ability to ameliorate antioxidant enzymatic defense system and reduce lipid peroxidation product in brain, liver and lungs of experimental animals in a tissue and a dose- dependent manner (Shinohara *et al.*, 2002). Propolis is rich with essential elements, such as Ca2+, Zn2+, Cu2+, Mg2+, Fe2+, Ni2+ and Mn2+ (Haro *et al.*, 2002), which may be responsible for reactivating antioxidant enzymes by providing optimum trace elements.

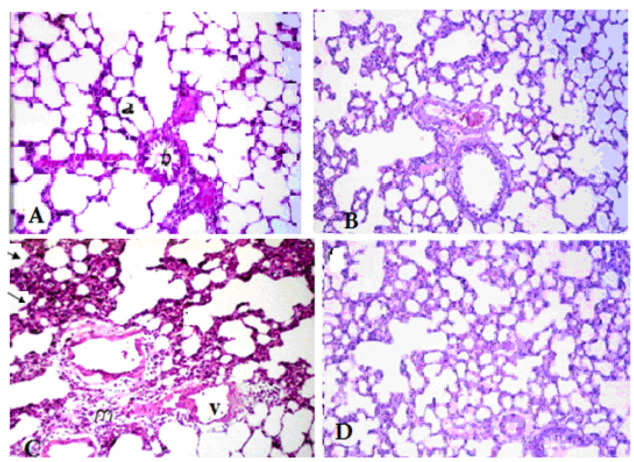

Fig. 6: Representative photographs of histological sections of lung tissues of normal and *S aureus* infected rat experimental groups (H & E staining, 400x). (A) lung of normal rats showing thin-walled alveoli (a) with a single layer of pneumocytes and a thin layer of connective tissue between the alveoli (ct) and bronchioles (b) are lined by ciliated epithelium. (B) lung section of rat ingested with propolis aqueous extract showing normal architecture of lung. (C) Lung section of *S aureus* infected rat-showing thickening of alveolar walls (arrows), infiltration of inflammatory immune cells with edema (m) surrounding the dilated blood vessel (v). (D) Lung section of rat lung infected with *S aureus* and co-administered with propolis aqueous extract showing more or less normal architecture of lung

The present study also showed a marked increase in the serum immunologic proinflammatory cytokine, TNF-α, in rats infected with methicillin-resistant *S aureus* compared with the normal ones. The over production of such cytokine may play a fundamental role in lung injury induced by *S aureus* infection. Previous studies reported that *S aureus* produces toxins, including Panton-Valentine leukocidin (Labandeira-Rey *et al.*, 2007), α -toxin, (Wardenburg *et al.*, 2007) and protein A (Gomez *et al.*, 2004) that is implicated in the pathogenesis of the lungs (Szmigielski *et al.*, 1999). These toxins can activate polymorphonuclear leukocytes (PMNs), to release potent proinflammatory cytokines including TNF-α and to produce reactive oxygen metabolites, causing lung inflammation and injury (Szmigielski *et al.*, 1999; Dragneva *et al.*, 2001; Gomez *et al.*, 2004; Ratner *et al.*, 2006; Cheng *et al.*, 2009; Holzinger *et al.*, 2012).

Simultaneous treatment of methicillin-resistant *S aureus* infected rats with aqueous extract of red propolis, successfully attenuated *S aureus* induced overproduction of inflammatory cytokine, TNF-α, suggesting that the potential effect of this propolis against lung damage induced by *S aureus* infection may be related to its immunomodulatory and anti-inflammatory beneficial impacts. The immunomodulatory and anti-inflammatory of different propolis including Saudi propolis were previously documented (Hu *et al.*, 2005; Ramos and Miranda, 2007; Sforcin, 2007; Saleh *et al.*, 2013). The anti-inflammatory effect of propolis may be related to the flavonoid and the phenolic compounds, (Chirumbolo, 2011) which have been reported as predominant biologically-active compounds in Saudi propolis (Abd El-Mawla *et al.*, 2011).

The present study illustrated a significant increase in the angiogenic biomarker, VEGF in the serum of rats infected with *S aureus*. Previous published data revealed that production of various inflammatory mediators such as cytokines, and chemokines, stimulates VEGF expression by immune and inflammatory cells (Verheul *et al.*, 2000; Lingen, 2001). Up-regulation of VEGF has been demonstrated to be a major contributor to angiogenesis in terms of stimulating vasculogenesis and angiogenesis (Ding *et al.*, 2004). It has the principle role in restoring oxygen supplementation to tissues when blood circulation is inadequate (Prior *et al.*, 2004). However, previous studies demonstrated that that production of TNF-α and VEGF was significantly linked. Both VEGF and TNF- α can promote a procoagulant state by inducing over expression of tissue factor on monocytes and/or endothelial cells (Clauss *et al.,* 1996; Mechtcheriakova *et al.*, 2001). Over expression of tissue factor is thought to play a pronounced role in multiorgan system failure in acute injury (Mechtcheriakova *et al.*, 2001). This suggests the possibility that TNF- α and VEGF induced by *S aureus* might act synergistically to induce lung tissue damage and/or systemic organ dysfunction. Therefore, the inhibition of VEGF production is considered an important therapeutic target against organ dysfunction.

Co-administration of propolis extract to *S aureus* infected rats, markedly reduced the dramatic increase in the angiogenic biomarker in their sera, suggesting its beneficial anti-angiogenic action. The anti-angiogenic effect of the used propolis may be related to the anti-inflammatory of its polyphenolic compounds. A previous investigation revealed the role of polyphenols from different sources of propolis in suppressing the inflammatory and angiogenic factors induced by atherosclerotic lesions (Daleprane *et al.*, 2012a). Also a previous study demonstrated that polyphenols of red propolis have antiangiogenic impact (Daleprane *et al.*, 2012b). The antiangiogenic effect of polyphenols of red propolis may related to its ability to attenuate the formation of new blood vessels, decrease the differentiation of embryonic stem cells into CD31-positive cells and reduce migration and sprout of endothelial cells (Daleprane *et al.*, 2012b). The authors added that the polyphenols of red propolis could also inhibit hypoxia- or dimethyloxallylglycine-induced VEGF expression. In addition, it has been suggested that dietary ingestion of polyphenols can suppress the incidence of organ damage which accompanied with over expression of VEGF and angiogenesis (Oak *et al.*, 2005).

It was found that the production of inflammatory cytokines by bacterial various virulence factors and the subsequent formation of reactive oxygen species (ROS) could induce host cell apoptosis through promoting genotoxicity (Simon *et al*., 2000, Prasad *et al*., 2011), resulting in tissue damage. DNA is an important target of oxidative damage by ROS induced by bacterial infection (Prasad *et al*., 2011).

The comet assay, or a single-cell gel electrophoresis, is a sensitive and a simple assay for detecting DNA damage at the level of individual cells (Singh *et al.*, 1988). The degree of DNA migration can be correlated to the extent of DNA fragmentation occurring in each single cell, which is associated with apoptosis (Tice and Strauss, 1995). With an increasing number of breaks, DNA pieces migrate freely into the tail of the comet and in extreme cases (the apoptotic cell), the head and tail are well separated. Tail length, tail moment as well as the percentage of total DNA in the tail, reflect DNA damage (Collins *et al.*, 1996).

The current study revealed that infection of rats with *S aureus* induced lung DNA fragmentation as documented by significant increases in the tail length, tail DNA% and tail moment in the lung tissues of infected rats compared with normal ones. The potential DNA-damaging effect of *S aureus* infection was verified by a previous experimental study (Prasad *et al.*, 2011). The present study suggests that the ability of this bacteria to induce lung - DNA damage may be related to lipid per oxidation and oxidative stress. ROS are known to react with DNA molecules, causing damage to both purine and pyrimidine bases as well as the DNA backbone (Martinez *et al.*, 2003). In addition, MDA, a major product of lipid peroxidation, is a mutagenic and carcinogenic compound. This compound reacts with DNA to form adducts to deoxyguanosine, deoxyadenosine, and deoxycytidine (Marnett, 2002; Niedernhofer *et al.*, 2003). DNA damage resulting from any of these probable mechanisms may trigger signal transduction pathways leading to apoptosis or cause interferences with the normal cellular processes, thereby causing cell death (Marnett, 2002; Diep *et al.*, 2010). Treatment of *S aureus* infected rats with propolis extract, effectively ameliorated lung DNA damage compared with infected untreated ones. This result is supported by a similar finding of Saleh (2012) who stated that an aqueous extract of propolis could ameliorate DNA damage induced in rat livers by octylphenol. Also, some authors reported that an aqueous extract of propolis could modulate DNA damage generated by dimethylhydrazine in colon cells (de Lima *et al.*, 2005).

Apoptosis represents a key event after oxidative DNA damage (Plesca *et al.*, 2008). The data in the current study showed a marked increase in the activity of the apoptosis biomarker, caspase 3, in the lung tissue of *S aureus* infected rats, suggesting that apoptosis might contribute to this pathogen-induced DNA damage. Treatment of *S aureus* infected rats with propolis extract, beneficially down-modulated the increase in lung caspase 3. This result may indicate that propolis extract mediated protection against *S aureus* -induced lung damage through its strong antiapoptotic effect. The anti-apoptotic mechanism of the used propolis may be related to its ability to inhibit DNA damage induced by *S aureus* infection. Propolis has been demonstrated to play an important role in preventing the oxidative stress, apoptosis and necrosis induced by lead (El-Masry *et al.*, 2011). Also Saleh (2012) reported that aqueous extract of propolis could ameliorate cell apoptosis induced by octylphenol in rat livers.

The damaging effect of *S aureus* infection to rats was also confirmed by histopathological examination of lung tissue. Control lung from normal rats showed normal alveolar architecture in which thin-walled alveoli with a single layer of squamous epithelium and a thin layer of connective tissue between the alveoli were observed. Propolis extract administration to normal rats did not alter lung histoarchitecture, while lungs of *S aureus* infected rats showed thickening of alveolar walls and infiltration of inflammatory immune cells with edema Similar lung histopathological result was obtained by some authors (Bubeck Wardenburg *et al.*, 2007). Co administration of propolis extract to infected rats showed more or less normal lung architecture. Similarly, a previous study revealed that propolis administration did not alter lung histological picture and showed protective effect against lung histopathological damage caused by inflammatory cytokines and oxidative stress in response cigarette smoke (Lopes *et al.*, 2013). Also, it was reported that propolis showed protective effects against liver and kidney histopathological damage in experimental animal models by its inhibitory action against lipid peroxidation and by its free radical scavenging ability (Bhadauria *et al.*, 2008; El-Kenawy *et al.*, 2014).

CONCLUSION

The present results suggest that prophylactic supplementation of red propolis aqueous extract may be beneficial against oxidative stress, inflammation oxidative DNA damage and apoptosis induced lung damage in response to methicillin-resistant *S aureus* infection. The beneficial effect of the used propolis extract against *S aureus* induced lung damage may be related to the antioxidant the anti-inflammatory, the immunomodulatory and the antiapoptosis of its active constituents.

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