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Therapeutic effect of propolis on *Staphylococcus aureus* induced oxidative stress in kidney of BALB/c mice. A biochemical and histopathological study

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Due to emerging drug resistance in pathogenic organisms, most of the second generation antibiotics are not effective in controlling the disease. As a consequence, the dosage and duration of drug intake has increased leading to drug induced toxicity and various side effects. A large number of natural products are being reported to ameliorate the toxicity and oxidative stress caused by antibiotics. Here, we explored the antioxidative potential of honey bee product propolis alone as well as in combination with antibiotics in *Staphylococcus aureus* infected BALB/c mice. For experimental design, mice were divided in to seven groups and decapitated after experimental period. Kidney was excised, homogenized and then used for different biochemical and histopathological estimations. Results observed after treatment with propolis and antibiotics were compared with those of *S. aureus* infected group. Results showed increase in lipid peroxidation, decrease in reduced glutathione levels and antioxidant enzymes such as; catalase, superoxide dismutase, glutathione-S-transferase, glutathione peroxidase and glutathione reductase. On the contrary, treatment with propolis, led to reduction in levels of LPO and increase in activities of antioxidant enzymes. Also, histopathology of kidney and all kidney function enzymes were restored to near normal.

Keywords: Amoxicillin, Ampicillin, Antioxidative potential, Apitherapy, Bee glue Multidrug-resistance (MDR)

Staphylococci are a Gram positive, non motile, non-spore forming commensal organisms. It is commonly cited as a major pathogen found under hospital settings and is a common opportunistic bacterium due to combination of its toxin-mediated virulence, invasiveness and antibiotic resistance[1]. Moreover, it is capable to survive within phagocytic cells both in polymorphonuclear leukocytes (PMN) and monocytes. Occurrence of multidrug-resistant (MDR) strains of this organism necessitates research for new classes of antimicrobial agents[2]. Since most drugs have lot of side effects associated with the duration of treatment, other alternate natural products need to be evaluated for their therapeutic efficacy. Apitherapy which involves application of honey bee products is emerging as a promising line of treatment in this direction[3-6].

Propolis also called 'Bee Glue' is the most remarkable bee product because of its wide range of biological and pharmacological potentialities. It is collected and brought by worker honey bees from various plants resinous secretions[7]. After collecting it, bees mix it with their salivary/enzymatic secretions and use it as a hive defensive material. The biological properties of propolis depend upon its chemical constituents[7-9] (polyphenols, terpenoids, steroids and amino acids), geographical regions and seasons[10-13]. It has been reported that pathogenic microorganisms cause damage in body by buildup of cellular oxidative stress[14,15]. It is initiated by free radicals and causes protein and DNA damage along with lipid peroxidation[16-19].

Propolis has been used in traditional medicine from ancient times in many countries[20,21]. It possesses various biological and pharmacological activities such as antioxidative[22-24], antibacterial[25-28], antiviral[13,29], antiinflammatory[29], anticancer[29,30], antifungal[29,31], immunomodulatory[13,32], therapeutic/cosmetic[33] and also as a feed additive in poultry nutrition[34,35]. These health benefits attributed due to its pharmacological and biological properties have attracted the interest of researchers and scientists[36].

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Much research has been done to study antioxidative properties of propolis under *in vitro* conditions. However, systematic studies on ameliorative effects of propolis using animal model are still lacking. Hence, in this study, we investigated the therapeutic potential of propolis along with antibiotics in *Staphylococcus aureus* infected Balb/c mice through biochemical and histopathological studies.

Materials and Methods

Collection and preparation of the propolis extract

Propolis of *Apis mellifera* was collected from Langstroth hives placed in the field of *Brassica campestris* at an apiary in Chandigarh, India. It was collected by scrapping it from the frames with the help of the hive tool. For extraction of propolis, a sample of 10 g was cut into small pieces; ground and extracted using ethanol[37]. The volume was made to 40 mL and kept for 5 days with occasional shaking. It was filtered through a Whatman No.41 filter paper and then dried.

Microorganism

Staphylococcus aureus (MTCC-1144) was procured from CSIR-Institute of Microbial Technology, Chandigarh, India. It was grown in BHI (Brain Heart Infusion) broth and maintained in BHI agar for further experiments. The organism was checked biochemically prior to storage at –30°C.

Animal model

BALB/c strain (5-6 wk old, male or female, weighing 25-30 g) of mice were used as experimental model. Mice were obtained from Central Animal House, Panjab University, Chandigarh, India and fed with a standard pellet diet; purchased from Ashirwad Industries, Kharar (Punjab) and water. Mice were kept in animal house at temperature (25±2°C) under 12 h light/dark cycle. Treatment was according to the guidelines of institutional ethical committee for the purpose of control and supervision of experiments on animals. It was approved by Institutional Animal Ethics Committee (PU/IAEC/S/14/136) of Panjab University, Chandigarh, India.

Experimental design

Selected animals were grouped seven groups with eight mice in each group as follows: Gr. I, Control mice administered with normal saline only (negative control); Gr. II, Mice infected with *S. aureus* (0.2 mL once, intra-peritoneal injection of 5×10^6 CFU/mL) positive control. Gr. III, Mice infected with *S. aureus* and given propolis extract (250 mg/kg body wt.) everyday for 15 days; Gr. IV & V, Mice infected with *S. aureus* and given antibiotic (ampicillin and amoxicillin, respectively @250 mg/kg body wt.) everyday for 15 days; similarly Gr. VI & VII, Mice infected with *S. aureus* and given ampicillin/amoxicillin and propolis extract dosages as above with a difference of two hours, everyday for 15 days.

Selection of propolis and antibiotic dose

Different doses were tested for propolis and antibiotics i.e. 50, 150, 250 and 350 mg/kg body wt./day. After studying significant biochemical alterations, the dose of 250 mg/kg body wt./day was selected for both propolis as well as antibiotics. The antibiotics were not effective at lower dose against *S. aureus*, hence decided the similar dosage level.

Separation, homogenization of kidney tissue and Biochemical studies

Staphylococcus aureus infected mice were sacrificed on 5th day as this was the peak day of infection while other groups were sacrificed immediately after 15th day by decapitation. Kidney tissue was excised from mice of different experimental groups, washed with cold normal saline, homogenized in the ice-cold buffer containing 0.25 M sucrose, 1m M EDTA, and 1 mM Tris-HCl, pH 7.4. This homogenate was used for LPO and GSH estimation directly and was centrifuged at 1000 rpm for 30 min at 4°C. The supernatant was used for further biochemical estimation of GST, SOD, CAT GPx and GR.

Assay of kidney function tests

Mice from all the groups were sacrificed and blood was collected from jugular vein in the Eppendorf tubes. Blood was kept for 20 min at room temperature (25±2°C) and then centrifuged at 3000 rpm for 30 min. The collected serum was colorless and was used for biochemical assays of urea, uric acid and creatinine using kits from Reckon Diagnostics Pvt. Ltd., India.

Histopathological studies of Kidney

For histopathological studies, kidney tissue was dissected out from normal and *S. aureus* infected mice on 5th day and *S. aureus* infected mice treated with protectants on day 15. It was washed in saline and fixed in Bouin's fixative[38]. After standard processing, sections were cut using microtome and were stained using haematoxylin and then counterstained with eosin[39].

Statistical analysis

Data were expressed as mean ± standard deviation (SD) and the statistical significance of the data was evaluated by one way analysis of variance (ANOVA) using SPSS software version 20. Further, data was analyzed by Scheffe post-hoc analysis with Least Square Difference. A value of P <0.05 was considered to indicate a significant difference and $P \leq 0.01$ highly significant difference between groups.

Results

Body weight

Reduction was observed in body weight of the *S. aureus* infected mice (Gr. II) as compared to the normal mice (Gr. I). The decrease was from (26.88±0.46 to 19.76±0.31 g) and this was found to be statistically highly significant ($P \leq 0.0001$) (Table 1). Administration of bee product propolis, antibiotics (ampicillin and amoxicillin) alone and their combination (dosage as described under methodology) with propolis revealed their therapeutic potentiality in restoring the weight of *S. aureus* infected mice (Table 1). Experimental groups (Gr. IV & V) also showed significant increase in the body weight as compared to the positive control group (Gr. II). *S. aureus* infected+propolis+ ampicillin/amoxicillin treated groups (Gr. VI & VII) restored the values to near normal, which revealed therapeutic potentialities of the combinational therapy (Table 1).

Survival percentage

For observing the survival of animals, eight mice were taken in each group at start of the experiment. As said earlier, 5th day was the peak day of infection where the rate of survival was recorded for *S. aureus* infected mice which were near about 90.23±5.72% but, animal showed signs of weakness, loss of appetite, reduced body wt. and lethargic behaviour. On 15th day, only 12.5% mice survived. After treatment with propolis and antibiotics alone, mice showed signs of recovery. Number of animals survived after administration of propolis, antibiotics (ampicillin and amoxicillin) alone and their combination with propolis authenticate present studies (Table 2).

Table 1 — Observed body weight of BALB/c mice used in the present experiment

Experimental groups	Body wt.
Gr. I (Normal)	26.88±0.46
Gr. II (*S taphylococcus aureus* infected)	19.76±0.31*
Gr. III (*S. aureus* infected+ Propolis)	22.44±0.76^
Gr. IV (*S. aureus* infected+ Ampicillin)	23.04±0.71^
Gr. V (*S. aureus* infected+ Amoxicillin)	23.74±0.11^
Gr. VI (*S. aureus* infected+ Propolis+ Ampicillin)	24.86±0.84^
Gr. VII (*S. aureus* infected+ Propolis+ Amoxicillin)	25.86±0.31^

[All the values are expressed as mean± SD (n=5). N *vs.* I (*$P \leq 0.0001$, &$P \leq 0.001$), I *vs.* Treated groups (^$P \leq 0.0001$, %$P \leq 0.001$)]

Biochemical studies

Levels of lipid peroxides were assayed by measuring the end product i.e. malondialdehyde (MDA). It was observed to be 0.39±0.010 n moles/mg protein in kidney after infection with *S. aureus* on 5th day and a highly significant increase was found as compared to control group (0.22±0.008 n moles/mg protein). After treatment with 250 mg/kg/body wt./day of propolis for 15 days, there was significant reduction in LPO of propolis treated group as compared to infected group, but it was still higher than normal. There is no significant change in the level of LPO in Gr. IV & V as compared to Gr. III (Fig. 1A). Level of lipid peroxides in Gr. VI (0.24±0.008 n moles/mg protein) and in Gr. VII (0.22±0.03 n moles/mg protein) showed a significant decrease showing effectiveness of the combination of amoxicillin and propolis (Fig. 1A).

Level of GSH decreased highly significantly from 1.77±0.03 µ moles/mg protein in normal mice to 0.93±0.01 µmoles/mg protein in kidney of *S. aureus* infected mice, indicating oxidative stress. In ampicillin and amoxicillin treated groups significant difference was observed that is 1.18±0.02 & 1.34±0.03 µmoles/mg protein, respectively, while in Gr. VI & VII highly significant increase was observed where the value with Gr, VI was 1.44±0.01 µmoles/mg protein and in Gr. VII it was 1.57±0.02 µ moles/mg protein (Fig. 1B).

There was significant decrease in activity of SOD i.e. 9.76±0.19 units/min/mg protein in *S. aureus*

Table 2 — Survival percentage (8 mice were taken in each group at start of experiment)

Experimental Groups	BALB/c mice: 1st Day	BALB/c mice: 15th Day	Survival on 15th Day (%)
Gr. I (Normal)	8±0	8.12±001	100%
Gr. II (*Staphylococcus aureus* infected)	8±0	1.07±0.022	12.5%
Gr. III (*S. aureus* infected+ Propolis)	8±0	5.34±0.161	62.5%
Gr. IV (*S. aureus* infected+ Ampicillin)	8±0	4.78±0.210	50%
Gr. V (*S. aureus* infected+ Amoxicillin)	8±0	4.599±0.181	56.25%
Gr. VI (*S. aureus* infected+ Propolis+ Ampicillin)	8±0	7.12±0.192	87%
Gr. VII (*S. aureus* infected+ Propolis+ Amoxicillin)	8±0	7.79±0.211	96.25%

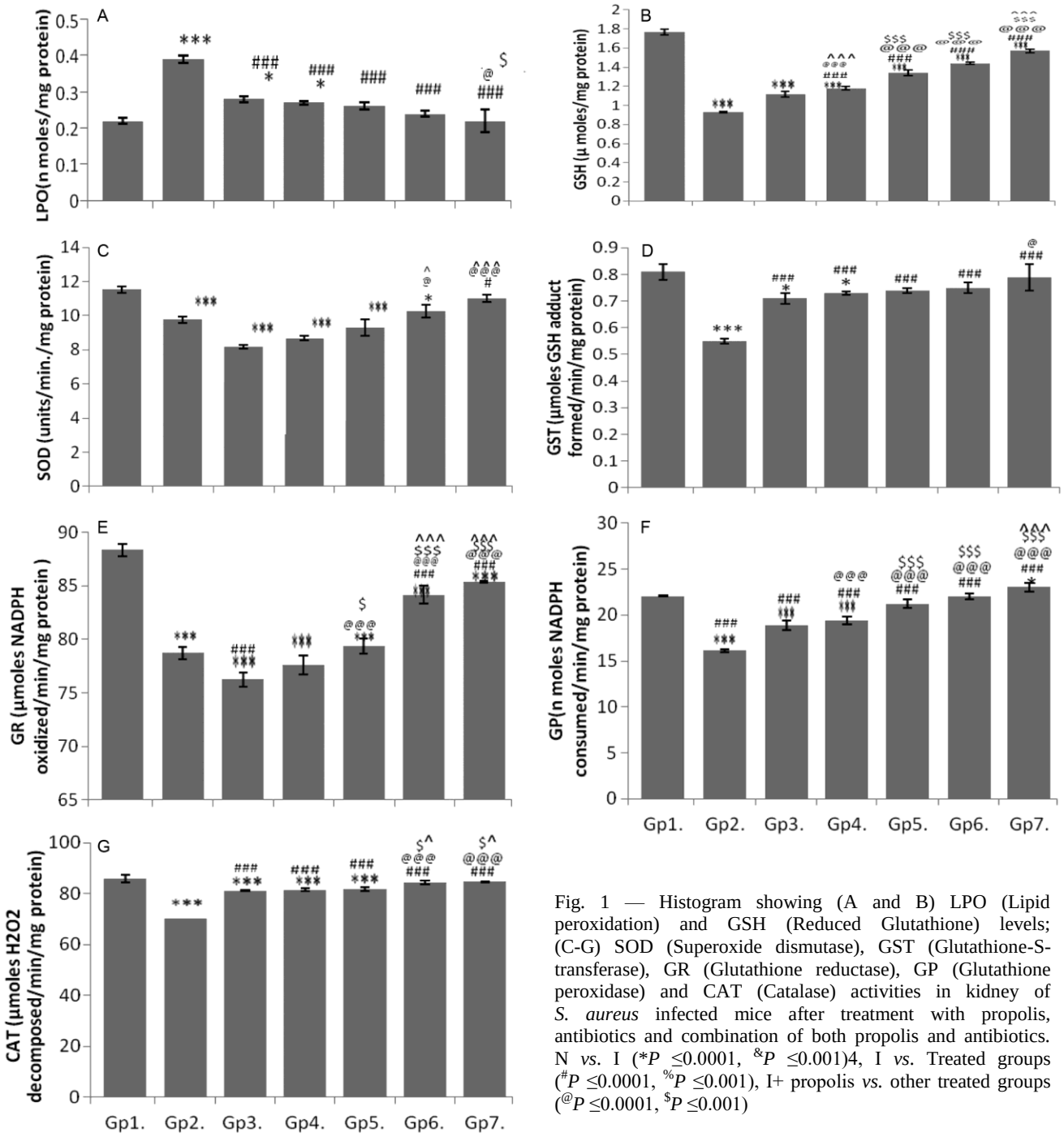

Fig. 1 — Histogram showing (A and B) LPO (Lipid peroxidation) and GSH (Reduced Glutathione) levels; (C-G) SOD (Superoxide dismutase), GST (Glutathione-S-transferase), GR (Glutathione reductase), GP (Glutathione peroxidase) and CAT (Catalase) activities in kidney of *S. aureus* infected mice after treatment with propolis, antibiotics and combination of both propolis and antibiotics. N *vs.* I (*$P \leq 0.0001$, $^{\&}P \leq 0.001$)4, I *vs.* Treated groups ($^{\#}P \leq 0.0001$, $^{\%}P \leq 0.001$), I+ propolis *vs.* other treated groups ($^{@}P \leq 0.0001$, $^{\$}P \leq 0.001$)

infected mice as compared to normal level of 11.53±0.18 units/min/mg protein. After treating with propolis there was significant increase in SOD activity which was 8.97±0.12 units/min/mg protein in Gr. III, while no significant difference was observed between Gr. IV and V. The activity with ampicillin and propolis was 10.27±0.38 units/min/mg protein and with amoxicillin and propolis it was 11.0±0.23 Units/min/mg protein which showed significant restoration of activity in combinational therapy (Fig. 1C).

GST acts as a detoxifying enzyme that conjugates electrophilic substrates to GSH containing thiol groups. During present study, activity of GST decreased significantly on 5th day after infection with *S. aureus*. This decrease was from 0.81±0.03 to 0.55±0.01 µmoles GSH adduct formed/min/mg

protein. Propolis treated group showed significant increments as compared to the infected group (Fig. 1D). The combinational groups i.e., Gr. VI (0.75±0.02 µmoles GSH adduct formed/min/mg protein) and Gr. VII (0.79±0.05 µmoles GSH adduct formed/min/mg protein) showed restoration in their values to near normal (Fig. 1D).

Further, during the present study the amount of GR was found decreased in case of infected group as compared to normal group. It was 88.35±0.54 µmoles NADPH oxidized/min/mg protein in normal group and 78.72±0.60 µmoles NADPH oxidized/min/mg protein in the infected group (Fig. 1E). In propolis treated group (Gr. III), the value was 76.25±0.64, while in Gr. IV and Gr. V it was 77.62±0.89 and 79.37±0.69, respectively. Further, Gr. VI and VII showed highly significant increase as compared to Gpr. IV and V. This revealed that the combinational therapy of propolis along with antibiotics restored the activity to near normal.

A significant decline in the level of GPx was observed after *S. aureus* infection as compared to normal and the decrease was observed from 22.06±0.06 to 16.12±0.18 n moles NADPH consumed/min/mg protein in kidney (Fig. 1F).

Catalase (CAT) is composed of four identical monomer units, each containing a heme group at the active site. Its main function is attributed to its degradation activity which degrades hydrogen peroxide to water. In the present study, *S. aureus* infection caused highly significant decrease in CAT activity (70.11±0.11 µmoles H_2O_2 decomposed/min/mg/protein) indicating increased levels of H_2O_2 which suggested oxidative stress due to *S. aureus* infection as compared to normal group 85.94±1.51 µmoles H_2O_2 decomposed/min/mg protein. Propolis treated group showed significant increase in catalase activity as compared to infected group *i.e.* 81.14±0.20 µmoles H_2O_2 decomposed/min/mg protein, while Gr. VI and VII showed restoration activity to near normal mice (Fig. 1G).

Kidney function tests

With respect to working of kidney, levels of urea, uric acid and creatinine were studied in serum samples of different groups of mice using commercially available kits. Level of urea, uric acid and creatinine showed significant increase in case of infected group as compared to the normal group (Table 3). The levels are; urea (46.32±1.58 to 85.81±3.37), uric acid (4.09 ±0.204 to 8.96±0.86) and creatinine (0.44±0.03 to 0.84±0.04 mg/dL). The disturbance observed in serum parameters levels indicated kidney damage caused by *S. aureus* infection. Treatment with propolis, ampicillin, amoxicillin and combination of propolis and antibiotics against infection of *S. aureus* in the present studies caused significant decrease in the levels of urea, uric acid and creatinine to near normal (Table 3).

Histopathological studies of Kidney

Histopathological analysis of kidney tissue of all experimental groups were done with the aim to determine the ameliorative effect of propolis in combination with standard antibiotics that is, ampicillin and amoxicillin against *Staphylococcus aureus* infection in BALB/c mice.

Kidney is a vital organ which is responsible for selective re-absorption, homeostasis, maintaining blood volume, blood pH and erythropoieses. To study the effects of propolis alone and in combination with antibiotics (ampicillin and amoxicillin) on histology of *S. aureus* infected kidney tissue, animals were divided in seven groups as shown under methodology. Histology of normal mice kidney (Gr. I) revealed the typical organization consisting of inner medulla and outer cortex (Fig. 2A i.e. under various magnifications). Medullary region consisted of renal pyramid and cortex region comprised of small spherical bodies called renal corpuscle which further comprise of two parts i.e. Glomerular and Bowman's capsule. Severe damage and disorganization of tubules was observed in *S. aureus* infected (Gr. II) kidney. Glomerular constriction, ruptured capsular wall, necrotic changes

Table 3 — Kidney function test

KFT	Gr. I (Normal)	Gr. II (*S. aureus* infected)	Gr. III (*S. aureus* infected+propolis)	Gr. IV (*S. aureus* infected+ ampicillin)	Gr. V (*S. aureus* infected+ amoxicillin)	Gr. VI (*S. aureus* infected+propolis+ ampicillin)	Gr. VII (*S. aureus* infected+propolis + amoxiicillin)
Urea (mg/dL)	46.328±0.707	85.818±1.508*	57.110±0.9729$^{*\#}$	55.55±0.471$^{*\#}$	55.324±0.819$^{*\%}$	48.104±0.508$^{\#@}$	44.568±0.576$^{\#@}$
Uric acid (mg/dL)	4.09± 0.0925	8.96±0.386*	5.88±0.163$^{\#\%}$	5.132±0.083$^{\#}$	4.62±0.199$^{\#}$	3.952±0.361$^{\#\%}$	3.386±0.197$^{\#@}$
Creatinine (mg/dL)	0.436±0.0156	0.838±0.017*	0.554±0.01$^{\#\%}$	0.486±0.009$^{\#}$	0.4500±0.020$^{\#\%}$	0.4380±0.033$^{\#\%}$	0.420±0.011$^{\#\%}$

[All the values are expressed as mean ± SD (n=5). N *vs*. I ($^{*}P \leq 0.0001$, $^{\&}P \leq 0.001$), I *vs*. Treated groups ($^{\#}P \leq 0.0001$, $^{\%}P \leq 0.001$), I+ propolis *vs*. other treated groups ($^{@}P \leq 0.0001$, $^{\$}P \leq 0.001$)]

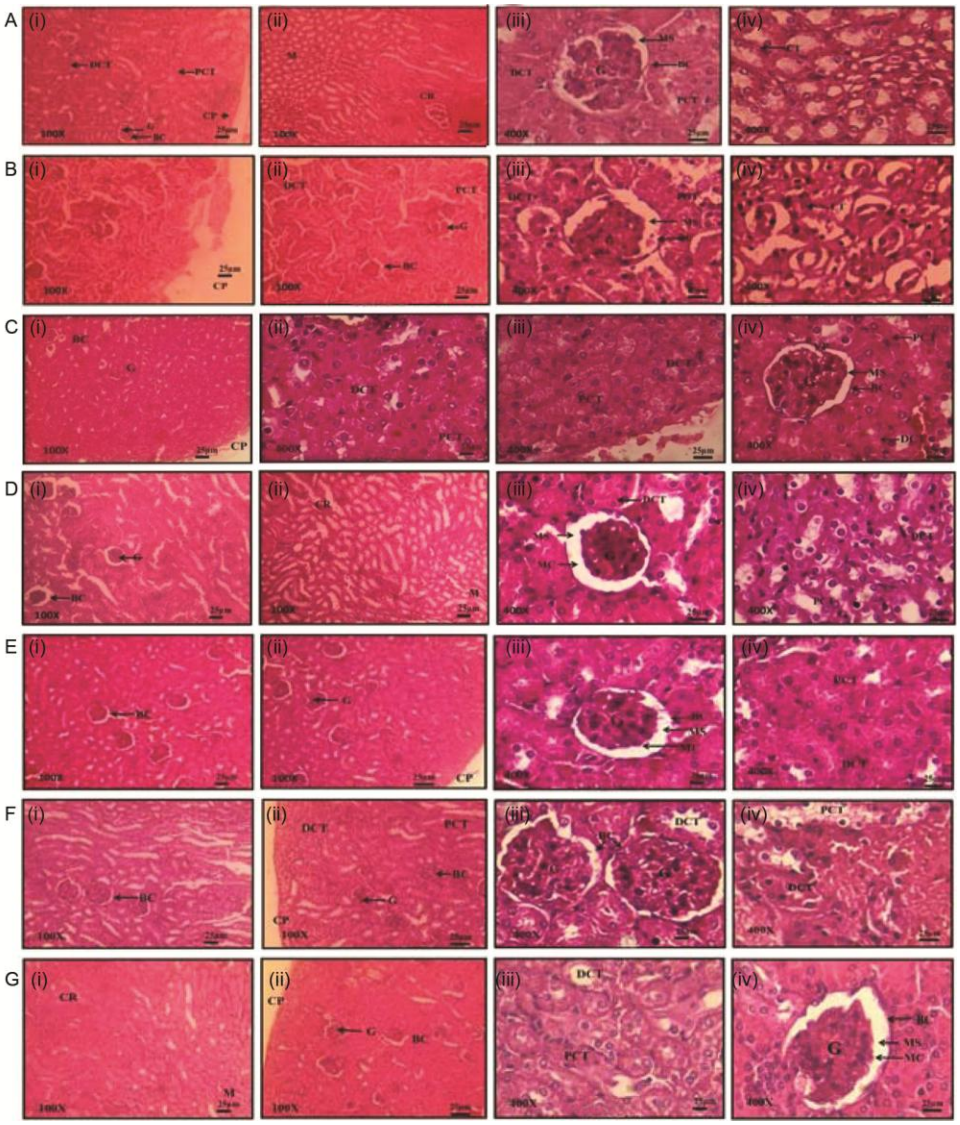

Fig. 2 — Histopathology of the kidney. Light micrographs of the kidney sections from different treatment groups. The numbers on the images represent different treatment groups. (A) control mouse kidney sections showing normal kidney architecture; (B) *S. aureus* infected kidney sections showing severe damage and disorganization of tubules, glomerular constriction, ruptured capsular wall; (C) *S. aureus* infected + propolis treated group showing some signs of recovery after propolis treatment; (D & E) ampicillin and amoxicillin treated group showing clearly distinguishable cortex and medulla and intact capsular wall; and (F & G) *S. aureus* infected+ propolis+ ampicillin and *S. aureus* infected+ propolis+ amoxicillin treated groups showing regular kidney morphology. (i, ii, iii & iv indicate light micrographs under various magnifications; 100 & 400X in different sections)

in the glomeruli, convoluted renal tubule and a severe damage to the epithelium of renal capsule, increase in the mesangial space, abnormal proliferation of mesangial cells, loss of brush border of the PCTs were also evident in *S. aureus* infected group (Fig. 2B). The above mentioned necrotic changes may be responsible for the renal failure as also seen from kidney function tests.

Histology of kidney of *S. aureus* infected+ propolis treated group (Gr. III) revealed that morphology of the brush border cells recovered, but the renal capsule was still found to be ruptured with some vacuolation (Fig. 2C). In ampicillin (Fig. 2D) and amoxicillin (Fig. 2E) treated group, cortex and medulla were clearly distinguishable, capsular wall was intact as compared to the propolis treated group however, constriction of mesangial cells and disoriented morphology of the PCTs, DCTs and CTs were still there.

The Transverse section (TS) of Gr. VI & VII exhibited regular kidney morphology with intact renal

capsular wall, cortex and medulla were distinguishable, increased mesangial cell proliferation, reduction in the mesangial spaces and the morphology of PCTs, DCTs was also found to be exactly similar to that of the normal group (Fig. 2 F & G). This indicates that propolis with ampicillin and amoxicillin showed higher therapeutic efficacy as compared to individual antibiotics and propolis treatment.

Discussion

Biochemical studies

Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidant molecules and enzymes. Free radicals can chemically interact with cell components such as DNA, proteins or lipids and steal their electrons in order to become stabilized. This in turn, destabilizes the cell component molecules which then seek and steal an electron from another molecule therefore, triggering a large chain of free radical reactions and hence, oxidative stress is a deleterious process which can be an important mediator of damage to cell structures, including lipids and membranes, proteins and DNA. Human body has several mechanisms to counteract this by producing exogenous and endogenous antioxidants[40]. Based on their activity, antioxidants are classified as enzymatic and non-enzymatic antioxidants. While enzymatic antioxidants[41,42] function by converting oxidized metabolic products in a multi-step process to hydrogen peroxide (H_2O_2) and then to water using cofactors, such as iron, zinc, copper and manganese. The non-enzymatic antioxidants functions by terminating free radical chain reactions. Examples of natural non-enzymatic antioxidants are some vitamins like vitamins A, E, C, polyphenols, flavonoids, carotenoids, glutathione, theaflavin, allyl sulfides, uric acid, curcumin, bilirubin and polyamines[43,44]. Antioxidants are lipophobic, predominantly found in the cytoplasm as well as lipophilic, present in the cell membranes[45].

Overproduction of free radicals has been related to nutritional, environmental and microbial stress due to bacterial, viral and fungal diseases[46]. In the present study, we have dealt with microbial stress due to *Staphylococcus aureus* which is an important cause of oxidative stress[47], as it has developed a mechanism to survive within the phagocytic cells both in polymorphonuclear leukocytes (PMN) and monocytes. Hence, it is difficult to deal with staphylococcal infections because phagocytosis is the major mechanism of defense against extracellular microbes.

The defense mechanisms in the form of endogenous antioxidants like reduced glutathione and enzymes such as GP, GR, CAT and SOD are active in reducing the level of free radical mediated oxidative stress as observed in present studies. However, these defense molecules and enzymes are not sufficient to control the burden of oxidative stress and its associated damage to lipids, proteins, cellular DNA. Therefore, in this direction many phytochemical have been found to play an important role as potential antioxidants and antimicrobials.

Now a day's bee products have acquired interest of researchers and scientists due to their pharmacological properties[48]. Amongst them, propolis is highly utilized by the bees as a chemical weapon for protection of their hive by preventing water infiltration, putrefaction of dead-intruders and maintaining local asepsis[48,49]. These antimicrobial activities of propolis are due to the presence of many phytochemical like flavonoids, CAPE, esters and some others substances, which act in synergism with each other to enhance the biological impact. However, HPLC or spectrophotometeric analysis should be done as the chemical composition of propolis is more complex and depends upon the bee species, the season in which it is collected, its botanical origin and the phytogeographical characteristics of the location where it was collected. Hence, in order to know about the exact active components of propolis responsible for various biological and pharmacological activities, HPLC or spectrophotometeric analysis should be done in further studies.

Propolis and antibiotics used in the present study might be inhibiting the penetration of *Staphylococcus* into phagocytic cells both in polymorphonuclear leukocytes (PMN) and monocytes and hence, eliminates its proliferation. In the present study when propolis was used along with antibiotics a synergistic behavior was observed. Although the mechanism behind this synergism is unknown till today, though there are some assumptions about it i.e. this combination leads to the formation of a complex which might be lysing the cell wall of bacteria, or it might be interfering with its cell wall synthesis and hence, directly or indirectly causing death of the bacteria[50].

Survival of the animals is the most important factor in experimental studies. Here, the *S. aureus* infected group showed signs of recovery after treatment with propolis and antibiotics alone as well as in their combinational therapy. As observed in present study, *S. aureus* infected mice showed heavy bacterial load in kidney tissues. However, a significant reduction in bacterial load was observed in combinational therapy used under present experiment. Hence, to assess the damage caused by *S. aureus* to kidney, the levels of urea, uric acid and creatinine were measured. These molecules are present in renal cells of healthy individual, hence raised levels of these molecules in blood indicates kidney damage caused by *S. aureus* infection. These raised levels of urea, uric acid and creatinine might be due to hindered glomerular filtration of urea, uric acid and creatinine[51,52]. Treatment with propolis, ampicillin, amoxicillin and combination of propolis and antibiotics against infection of *S. aureus* in the present studies caused significant decrease in the levels of urea, uric acid and creatinine as they were restored to near normal.

Present study revealed that after *S. aureus* infection, there is a significant increase in production of free radicals and tissue damage, as revealed by histopathological changes in kidney. The natural bee product propolis alone and in combination with antibiotics protected kidney tissue from derangements caused by the infection. Propolis along with amoxicillin treatment was found to be the most effective against induced infection, suggesting ameliorative as well as synergistic potential of propolis against bacterial infection. Amoxicillin was more effective as compared to ampicillin and propolis alone in the treatment of intraperitonial mouse infections when administered by oral routes[53]. The results revealed that the combination therapy of propolis along with antibiotics restored the antioxidant activity to near normal hence; it is suggested to be used in further clinical application.

Conclusion

In present studies, therapeutic potential of propolis was carried out in *Staphylococcus aureus* infected mice through biochemical and histopathological studies. Treatment with propolis and antibiotics alone as well as in combination ameliorates oxidative stress as well as histopathological alterations in kidney. Results obtained have shown increased efficacy of antibiotics when combined with propolis and hence higher therapeutic efficacy as compared to individual antibiotics and propolis treatment. This showed synergistic activity or complementation between propolis and antibiotics for fighting microbial infections. But, there is need to isolate and identify the specific active ingredients responsible for amelio-rative activities and to establish mechanism of action.

Conflict of interest

Authors declare no competing interests.

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