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Therapeutic effect of propolis on *Staphylococcus aureus* induced oxidative stress in spleen of Balb/c mice: A biochemical and histopathological study

Anita Rana[1#], Neelima R Kumar[1] and Jaspreet Kaur[2*]

[1]Department of Zoology; [2]Department of Biotechnology (UIET), Panjab University, Chandigarh 160014, India

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The aim of present study was to evaluate antioxidant effect of propolis alone as well as in combination with antibiotics; ampicillin and amoxicillin against *Staphylococcus aureus* induced oxidative damage in spleen, by using biochemical and histopathological methods. Infection was induced in mice with *S. aureus* ($5x10^6$CFU/mL i.p) and protective potential of propolis against infection was evaluated by administrating 250 mg/kg body weight of propolis every day for 15 days. Mice were killed after experimental period and spleen was excised, homogenized and then used for different biochemical and histopathological estimations. Results obtained showed that lipid peroxidation level increased significantly (P <0.05), while level of reduced glutathione and activity of antioxidant enzymes (GP, GR, GST, CAT, and SOD) were decreased in spleen of *S. aureus* infected mice, which were increased to normal level (P <0.05) in propolis and antibiotics combination treatment. Present study also revealed that *S. aureus* infection caused significant change in cellular architecture of spleen as revealed by histopathological changes which were also restored to near normal after treatment with propolis and antibiotics when used in combination. These findings suggest therapeutic potential of propolis against *S. aureus* induced oxidative stress and histopathological damage in spleen.

Keywords: Ampicillin, Antioxidant, Histopathology, Propolis, Spleen, *Staphylococcus aureus*.

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Introduction

Staphylococcus aureus is a Gram-positive, facultative anaerobic, round shaped bacterium. It is prominently a part of the microflora commonly found in the nose and on skin. About 20% of the human population is long-term carrier of *S. aureus*[1]. *S. aureus* is a causal agent of local infections which can in later stages develop into systemic infections such as bacteremia, sepsis in adults[2,3] and in children[4]. Local infections are associated with skin and soft tissue damage such as wound infections, skin infections, cellulitis, abscesses[5] and deep infections which include myositis[6], osteomyelitis[7], pericarditis[8], endocarditis[9], septic arthritis[10] and pneumonia[11]. World Health Organization reported that bacterial resistance to antibiotics was a major global health challenge[12]. Major group of bacteria that evolved mechanisms for antibiotic resistance was *Staphylococci* especially *S. aureus*.

It has developed resistance to most classes of antimicrobial agents because of its ability to bypass all barriers of the host defence system, since it possesses a wide spectrum of virulence factors[13,14]. Virulent factors include protein-A, lipases, collagenase, hyaluronidase, coagulase, hemolysins and a variety of proteins affecting biofilm formation[15]. Moreover, it has evolved a mechanism to reside within phagocytic cells; hence it is very difficult to deal with staphylococcal infections.

So in view of the above said line, there is an urgent need to find novel, non-antibiotic chemotherapeutics with marked anti-staphylococcal activity. As per the studies and research carried out herein, bee products; such as honey, bee-pollen, bee-venom, royal-jelly, bee-bread and propolis[16] show anti-staphylococcal chemotherapeutics. Here we are focusing on one bee product called propolis, also known as bee glue. It is a natural resinous substance produced from resin of flowers, leaves of trees, plant buds and exudates, then modified by addition of bee's salivary secretions and wax[16,17]. As a resinous substance, it is used in sealing cracks, filling cavities in the walls of bee hive, for reducing entrance during cold days, to keep moisture and temperature stable in the hive[18], for mummifying the intruders and thus, preventing their rotting. This is the reason why propolis is known as bee glue[19].

*Correspondent author
Email: jaspreet_virdi@yahoo.com
#Present address: Department of Bioscience (UIBT), Chandigarh University, Mohali 140413, India

Due to its chemical composition, biological and pharmacological properties, it has grabbed a great deal of attention of researchers all over the globe. It has been used in traditional medicines for centuries; while in recent years it has attracted attention as a valuable substance used in alternative medicine[20-27]. Some of its ingredients mainly, polyphenols and flavonoids exhibit high antimicrobial activity[28]. As a consequence of which, it is used as a hive disinfectant.

This study is aimed at determining the possible antioxidant effects of *Apis mellifera* honey bees produced propolis alone and in combination with ampicillin and amoxicillin against *S. aureus* induced oxidative damage in spleen, by using biochemical and histopathological methods.

Materials and Methods

Collection and preparation of propolis extract

Propolis of *A. mellifera* was collected from Langstroth hives placed in the field of *Brassica campestris* in an apiary in Chandigarh (India). It was collected by scrapping it from the comb frames with the help of the hive tool in the month of June-July, 2018. For the extraction of propolis, standard protocols of the authors' lab were followed[23]. Here crude sample (10 g) was cut into small pieces, ground and extracted using pure ethanol, and the volume was made to 40 mL. It was kept for 5 days with occasional shaking. It was filtered through a Whatman No. 41 filter paper and then dried[23].

Microorganism

S. aureus (MTCC-1144) was procured from Institute of Microbial Technology (IMTECH) 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^{0}C.

Animal model

The BALB/c strains of mice of either sex were used. 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 then kept in animal house and the treatment was according to the guidelines of institutional ethical committee for the purpose of control and supervision of experiments on animals were followed and the same were duly approved by Institutional Animal Ethics Committee with approval no. (PU/IAEC/S/14/136 dt. 14.06.2018), Panjab University.

Experimental design

For the experimental design, the animals were segregated into seven groups, each group comprising of six mice as follows: Group 1: control mice administered with normal saline only (negative control), Group 2: mice infected with *S. aureus* (0.2 mL once, intra-peritoneal injection of 5×10^6 CFU/mL) i.e. positive control group, Group 3: mice infected with *S. aureus* and given propolis extract (250 mg/kg b.w.) every day for 15 days, Group 4: mice infected with *S. aureus* and given antibiotic (ampicillin; 250 mg/kg b.w.) every day for 15 days, Group 5: mice infected with *S. aureus* and given antibiotic (amoxicillin; 250 mg/kg b.w.) every day for 15 days, Group 6: mice infected with *S. aureus* and given ampicillin and propolis extract dosages as above with a difference of two hours, every day for 15 days, Group 7: mice infected with *S. aureus* and given amoxicillin and propolis extract dosages as above with a difference of two hours, every day for 15 days.

Preparation of tissue homogenate and PMS

All animals used in the experiment were sacrificed by decapitation. The infected (untreated) group was sacrificed on the 5th day which was observed to be the peak day of infection, while other (treated) groups were sacrificed after 15th day of treatment. Spleen was removed aseptically in laminar air flow chamber and perfused immediately with sterile ice cold saline (0.89% NaCl) solution. The tissue was homogenized in autoclaved 100 mM potassium phosphate buffer (pH 7.4) containing 150 mM KCl to obtain 10% homogenate (w/v). Homogenate was used for lipid peroxidation and reduced glutathione assay. After this, the homogenate of spleen tissues was subjected to cold centrifugation at 10,000 x g for 30 minutes. The pellets were discarded and supernatant (post mitochondrial supernatant) was used for further estimation of GPx (Glutathione peroxidase), GR (Glutathione reductase), GST (Glutathione-S-transferase), SOD (Superoxide dismutase) and CAT (Catalase).

Biochemical estimation of oxidative stress related parameters in spleen

Estimation of lipid peroxidation (LPO)[29]

LPO was estimated by thiobarbituric acid-reactive substances (TBARS) assay, performed by

malondialdehyde (MDA) reaction with 2-thiobarbituric acid (TBA) Homogenate (0.1 mL) was added in 0.1 mL each of 150 mM Tris-HCl (pH-7.1), 1.5 mM ascorbic acid and 1.0 mM ferrous sulphate in a final volume of 1 mL and incubated at 37°C for 15 minutes. To this, 1 mL of 10% trichloroacetic acid (TCA) and 2 mL of 0.375% thiobarbituric acid were added and kept in boiling water bath for 15 minutes. The contents were centrifuged at 3000 rpm for 10 minutes and optical density was measured at 532 nm. The units were expressed as n moles of MDA formed per mg protein.

***Estimation of reduced glutathione (GSH)*[30]**

Exactly 1 mL of sulphosalicyclic acid was added to 1 mL of homogenate and centrifuged at 1200 rpm for 5 minutes. Collected 0.5 mL of supernatant and to which 4.5 mL of DTNB (Ellman'sreagent) was added. Allowed to stand for 2 minutes and then recorded the absorbance at 412 nm. The molar extinction coefficient of mercaptobenzoic acid is $1.36x10^3$ M/cm at 412 nm. The GSH content was measured as µMoles GSH/mg protein.

***Determination of superoxide dismutase (SOD) activity*[31]**

The reaction mixture containing 1.2 mL of solution A (50 mM sodium carbonate in 0.1 mM EDTA buffer, pH 10.8), 0.5 mL solution B (96 µM NBT) and 0.1 mL of solution C (0.6% Triton X-100) were incubated at 37°C for 10 minutes. Reaction was initiated by adding 0.1 mL of 20 mM hydroxylamine HCl (pH 6). The rate of NBT dye reduction by O-2 anion generated due to photoactivation of hydroxylamine HCl was recorded at 560 nm for 3 minutes for blank. Then 0.1 mL PMS was immediately added after addition of hydroxylamine HCl to the reaction mixture. After mixing thoroughly, 50% inhibition in the rate of NBT reduction by SOD present in the enzyme source was recorded at 560 nm for 3 minutes. Change in absorbance (blue colour) was measured at 560 nm for 3 minutes at 30 seconds intervals.

***Estimation of catalase (CAT) activity*[32]**

The assay mixture was prepared of 2.9 mL of 12.5 mM H_2O_2 and 0.067 M phosphate buffer (pH 7.0) and 0.01 mL PMS (DDW for blank). The decrease in absorbance/30 sec at 240 nm was measured for 3 minutes. The activity of enzyme is expressed as µ moles of H_2O_2 decomposed/min/mg of protein, using 0.071 molar extinction coefficient of H_2O_2.

***Determination of glutathione-S-transferase (GST) activity*[33]**

PMS was incubated in 1 mL of 0.2 M phosphate buffer (pH-6.5), 0.1 mL of 20 mM 1-chloro-2, 4-dinitrobenzene (CDNB) prepared in 95% ethanol and 0.8 mL of DDW. After mixing thoroughly, incubation was carried out at 37°C for 5 minutes. To this, 0.1 mL of 20 mM GSH (dissolved in DDW) was added just before measuring the increase in absorbance/30 sec at 340 nm for 5 minutes. In case of blank, 2.9 mL phosphate buffer and 0.1 mL CDNB were mixed. The enzyme activity was expressed as µ moles/mg protein.

***Determination of glutathione reductase (GR) activity*[34]**

The reaction mixture was formed by mixing of 5 mL of GSSG, 5 mL of EDTA, and 5 mL of NADPH. To the 1 mL of reaction mixture, 100 µL of enzyme source was added. The enzyme activity was measured by recording the change in absorbance per minute for 3 minutes after 30 seconds intervals at 340 nm. One unit of enzyme activity was expressed as m moles of NADPH consumed/min/mg protein by using an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

***Determination of glutathione peroxidase (GPx)*[35]**

The reaction mixture contained 5 mL of 1 mM EDTA, 5 mL of 0.3 mM reduced glutathione, and 5 mL of 0.2 mM NADPH. To 1 mL of reaction mixture, 100 µL of enzyme source was added. After this, added 11.5 µL of yeast glutathione reductase. The decrease in absorbance at 340 nm for 3 minutes at 30 seconds intervals was noted. The activity of enzyme was calculated as m moles NADPH oxidized/min//mg protein.

Histopathological studies on spleen

For histopathological studies, spleen tissue was dissected out from normal and *S. aureus* infected mice on 5th day and from *S. aureus* infected and treated mice on 16th day. It was washed in saline and fixed in Bouin's fixative. After standard processing, sections were cut at 5 µ using microtome. Transverse sections were stained using haematoxylin and then counterstained with eosin.

Statistical analysis

Data was 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 the body weight of *S. aureus* infected mice (Group 2) as compared to the normal mice (Group 1). The decrease in body weight was from (26.88±0.46 to 19.76±0.31 g) and it was found to be statistically significant ($P \leq 0.0001$) (Table 1). Administration of 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). Positive control groups (Groups 4 and 5) also showed significant increase in body weight as compared to *S. aureus* infected (Group 2) group. *S. aureus* infected + propolis + ampicillin (Group 6) and *S. aureus* infected + propolis + amoxicillin (Group 7) treated groups restored the values to near normal, which revealed therapeutic potentialities of the combinational therapy.

Biochemical estimation of oxidative stress related parameters in spleen

Estimation of lipid peroxidation

Level of lipid peroxidation was assayed by measuring the end product i.e., MDA. It was observed to be 0.580±0.007 n moles/mg protein in spleen of *S. aureus* infected mice and 0.430±0.007 n moles/mg protein in control group. On propolis administration (250 mg/kg/b.w./day) for 15 days, significant reduction in lipid peroxidation was observed as compared to infected group, but it was still higher than normal. No significant change was observed in the level of LPO in groups 4 and 5 as compared to group 3 (Fig. 1). Level of lipid peroxides in group 6 (0.430±0.008 n moles/mg protein) and in group 7 (0.420±0.005 n moles/mg protein) showed a significant decrease proving effectiveness of the combination of antibiotics and propolis (Fig. 1).

Estimation of reduced glutathione (GSH)

Level of GSH decreased significantly from 1.548±0.043 µ moles/mg protein in normal mice to 0.858±0.012 µmoles/mg protein in spleen of *S. aureus* infected mice, indicating oxidative stress. Ampicillin and amoxicillin treated groups make a significant difference and the values observed were 1.274±0.0150 and 1.342±0.008 µmoles/mg protein respectively. In combinational treatment i.e. groups 6 and 7, highly significant increase was observed where the value with group 6 was 1.504±0.009 µmoles/mg protein and in group 7, it was 1.546±0.006 µ moles/mg protein (Fig. 2).

Determination of superoxide dismutase (SOD) activity

A significant decrease in activity of SOD (6.012±0.115 units/min/mg protein) was observed in *S. aureus* infected mice (group 2) as compared to normal control group (11.530±0.138 units/min/mg protein). Propolis treatment (group 3) led to a significant increase in SOD activity which was 7.758±0.060 units/min/mg protein, while no significant differences were observed between group

Table 1 — Body weight of control, *S. aureus* infected and treated BALB/c mice

Groups	Body weight
Normal	26.88±0.46
S. aureus infected	19.76±0.31*
S. aureus infected + Propolis	22.44±0.76^
S. aureus infected + Ampicillin	23.04±0.71^
S. aureus infected + Amoxicillin	23.74±0.11^
S. aureus infected + Propolis + Ampicillin	24.86±0.84^
S. aureus infected + Propolis + Amoxicillin	25.86±0.31^

All the values are expressed as mean±SD. N v/s I (*:$P \leq 0.0001$, &: $P \leq 0.001$), I v/s all treated groups (^: $P \leq 0.0001$, %: $P \leq 0.001$)

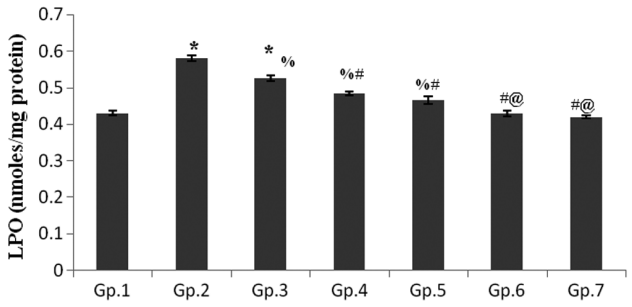

Fig. 1 — Effect on levels of lipid peroxidation (LPO) in spleen of *S. aureus* infected and different treatment groups of mice.

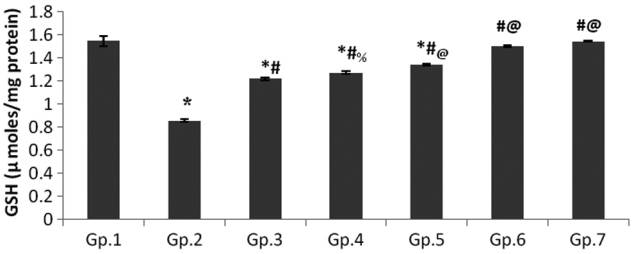

Fig. 2 — Effect on levels of GSH in spleen of *S. aureus* infected and different treatment groups of mice.

4 and 5. The activity with ampicillin and propolis (group 6) was 10.194±0.170 units/min/mg protein and with amoxicillin and propolis (group 7), it was 11.348±0.008 Units/min/mg protein which showed a significant restoration of activity in combinational therapy (Fig. 3).

Determination of glutathione-S-transferase (GST) activity

GST, which acts as a detoxifying enzyme, plays an important role in the oxidative stress related parameters. In the present study, after *S. aureus* infection, GST activity decreased significantly from 0.784±0.002 to 0.300±0.006 µmoles GSH adducts formed/min/mg protein. On propolis treatment a significant increase in GST activity was observed. Propolis when used along with antibiotics i.e., in group 6 (0.726±0.005 µmoles GSH adduct formed/min/mg protein) and in group 7 (0.758±0.004 µmoles GSH adduct formed/min/mg protein) led to significant restoration in the GST activity (Fig. 4).

Determination of glutathione reductase (GR) activity

Further, during the present study, GR activity was found to be decreased in the case of infected group as compared to normal group. It was 56.624±0.239 µmoles NADPH oxidized/min/mg protein in normal group and 38.942±0.178 µmoles NADPH oxidized/min/mg protein in the infected group (Fig. 5). In propolis treated group (group 3) the value was 46.910±0.121, µmoles NADPH oxidized/min/mg protein, while in groups 4 and 5 it was (48.134±0.113 and 49.728±0.238) µmoles NADPH oxidized/min/mg protein respectively. Further, when propolis was used along with antibiotics i.e., (group 6 and 7), highly significant increase in the GR activity was observed as compared to antibiotics alone treatment i.e., (groups 4 and 5). The combinational treatment i.e., (groups 6 and 7) validated the synergistic behaviour of propolis along with antibiotics.

Determination of glutathione reductase (GPx) activity

A significant decline in the GPx activity was observed in (group 2) as compared to normal group (group 1) and the decrease was from 10.974±0.236 to 6.702±0.231 n moles NADPH consumed/min/mg protein in spleen of *S. aureus* infected mice. Propolis and antibiotics treatment led to restoration of the enzyme activities when used in combination (Fig. 6).

Estimation of catalase (CAT) activity

This enzyme is mainly responsible for degradation activity of hydrogen peroxide to water and oxygen. In present studies, *S. aureus* infection caused highly significant decrease in CAT activity (29.090±0.356 µmoles H_2O_2 decomposed/min/mg/protein) indicating

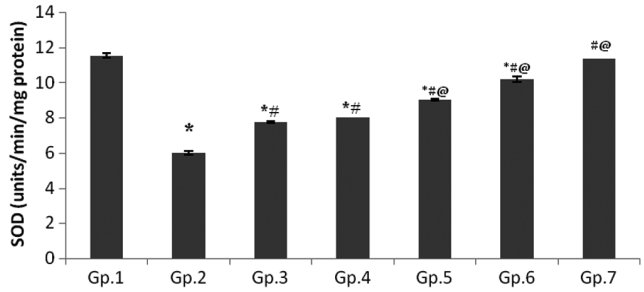

Fig. 3 — Effect on SOD activity in spleen of *S. aureus* infected and different treatment groups of mice.

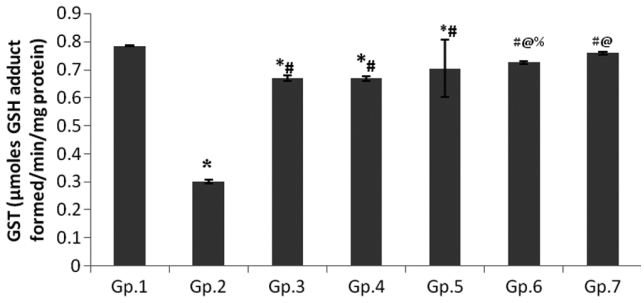

Fig. 4 — Effect on GST activity in spleen of *S. aureus* infected and different treatment groups of mice.

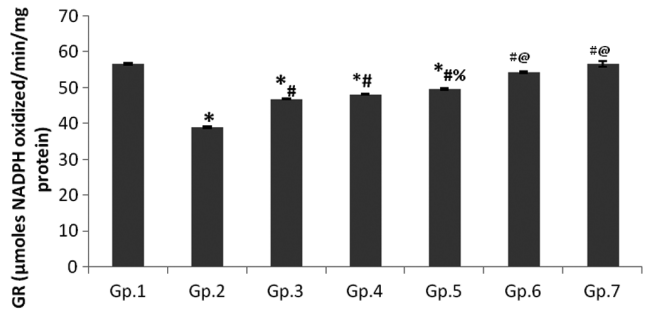

Fig. 5 — Effect on GR activity in spleen of *S. aureus* infected and different treatment groups of mice.

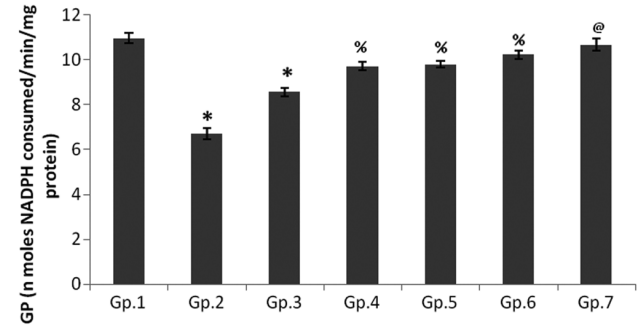

Fig. 6 — Effect on GP activity in spleen of *S. aureus* infected and different treatment groups of mice.

increased levels of H_2O_2 which suggested oxidative stress due to *S. aureus* infection as compared to normal group (75.294±0.401 µmoles H_2O_2 decomposed/min/mg protein). Propolis treatment led to significant increase in catalase activity as compared to the infected group i.e., 59.522±0.325 µmoles H_2O_2 decomposed/min/mg protein. Propolis when used along with antibiotics i.e., (groups 6 and 7) showed restoration activity of the enzyme (Fig. 7).

Histopathological study

Histological studies were performed for observing the micro-architecture of spleen in the normal, infected, and treated animals. In the present study, therapeutic potential of propolis was evaluated in *S. aureus* infected spleen of BALB/c mice. *Staphylococcus* is a usual member of microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is also a major human pathogen that causes a wide variety of infections[36] since it has evolved a mechanism to survive within phagocytic cells; hence it is an important causal agent of oxidative stress[37-39]. So to study the effect of *S. aureus* infection on the histology of spleen, the animals were divided into seven groups as described under the methodology.

The observations made on spleen micro-architecture led to the following observations. Spleen is a dark red elongated organ, roughly triangular in cross section and located in the left cranial abdomen within the omentum. The microscopic observations of normal spleen showed that it is comprised of 2 functionally and morphologically distinct compartments, the red pulp and the white pulp (Fig. 8 I). The red pulp is a blood filter that removes foreign material[40] and damaged erythrocytes. It is also a storage site for iron, erythrocytes, and platelets. In rodents, it is a site of hematopoiesis, particularly in

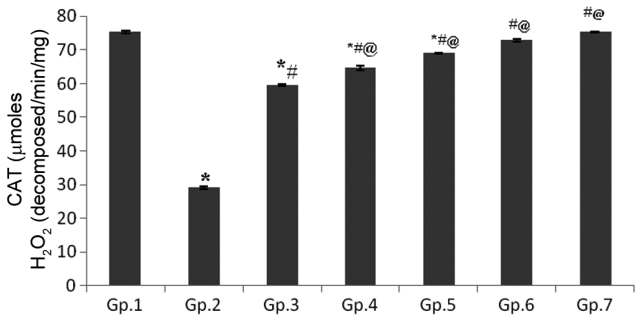

Fig. 7 — Effect on CAT activity in spleen of *S. aureus* infected and different treatment groups of mice.

fetal and neonatal animals. In earlier studies, it was made clear that the ability of red pulp compartment in not allowing foreign particles is by involving SLC11/Nramp family (natural resistance-associated macrophage protein) whose polymorphism is associated with susceptibility to a variety of pathogens[41,42].

Spleen is also the largest secondary lymphoid organ containing about one-fourth of the body's lymphocytes and initiates immune responses to blood-borne antigens. This function is charged to the white pulp which surrounds the central arterioles. White pulp is further composed of three sub-compartments: the periarteriolar lymphoid sheath (PALS), the follicles and the marginal zone which is a unique region of the spleen situated at the interface of the red pulp with the PALS and follicles. It is designed to screen systemic circulation for antigens and pathogens and plays an important role in antigen processing[40].

The spleen also plays a major role against pathogenic organisms like fungi, bacteria, and viruses. Its dysfunction leads to detrimental consequences with easy access to microbes and this facilitates more flourishing of microbes in body[43]. Microbe oriented systemic infections and distorted micro-architecture of splenic tissue is very common[44]. Some of the microbial infections progress with lymphoid or stromal splenic cell hyperplasia, sometimes followed by lymphoid atrophy and disorganization of spleen compartments[45-48].

Histopathological analysis of *S. aureus* infected spleen revealed that there were severe pathological changes and tissue injury with internal degeneration of red and white pulp, enlarged marginal zone, increase in the number of follicles and ruptured capsular wall (Fig. 8 II) as also observed under a previous study[37].

On the other hand, treatment with propolis and antibiotics, alone as well as in combination showed recovery of tissue damage and caused regeneration of red pulp with intact capsular wall, clear red and white pulp regions and marginal zone. In propolis alone treated group (Fig. 8 III) the capsular wall was regenerated but internal damage was not noteworthy as enlargement of white pulp and the marginal zone was observed. Infiltration of WBC's was also evident in the red pulp region while, treatment with ampicillin (Fig. 8 IV) and amoxicillin (Fig. 8 V) resulted in normal spleen architecture with a red pulp and white

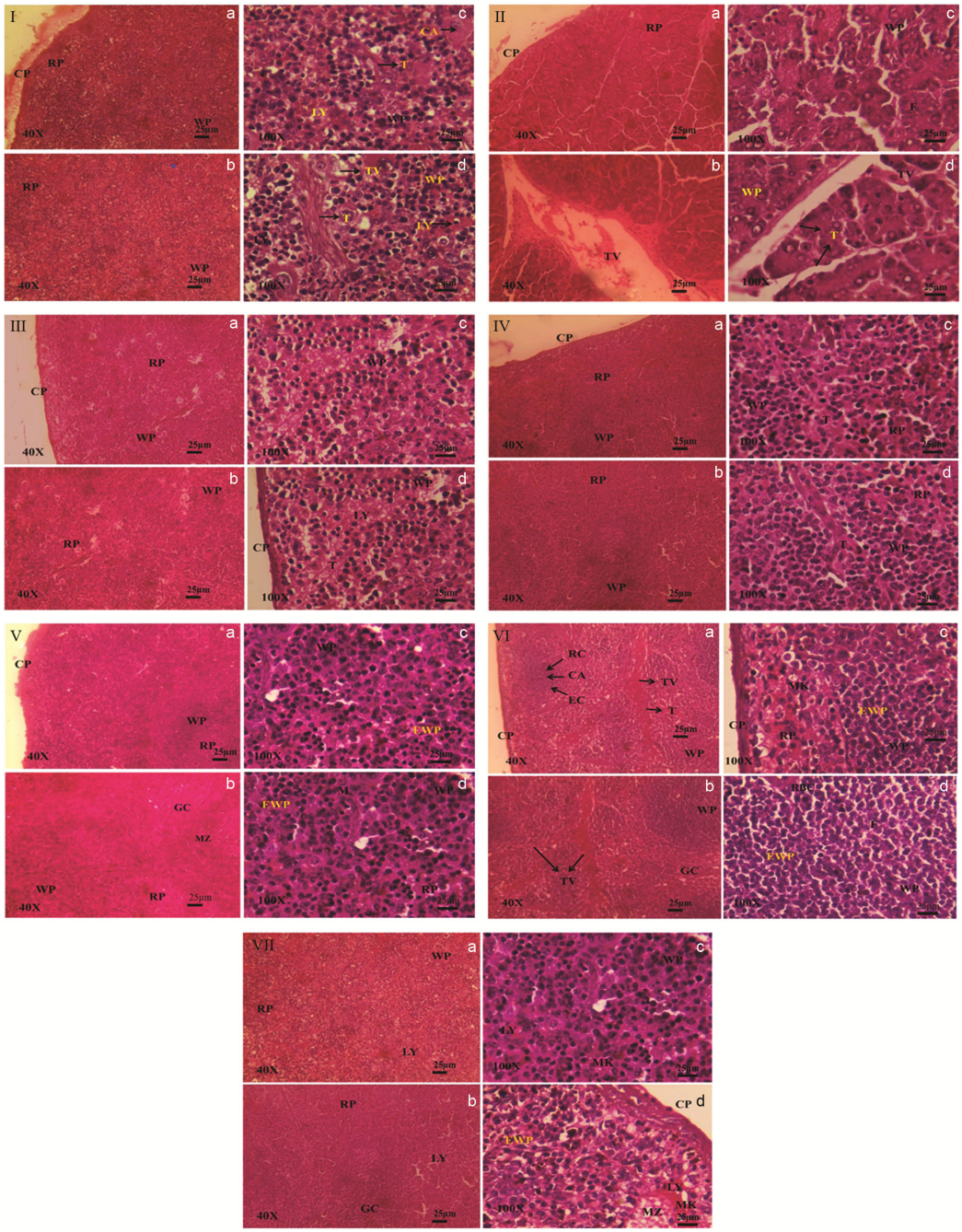

Fig. 8 — I-VII are histological representation of Spleen tissue after *S. aureus* infection and in different treatment groups.

pulp separated by marginal zone and intact capsular wall. The combination of antibiotics; ampicillin (Fig. 8 VI) and amoxicillin (Fig. 8 VII) respectively with propolis showed spleen architecture very similar to that of control group (Fig. 8 I) suggesting synergistic behaviour of ampicillin and amoxicillin with bee product propolis. It was also observed in our previous studies of *S. aureus* infection in kidney of Balb/c mice[49]. The combinational therapy also showed synergistic behaviour in case of cefixime and propolis in liver and spleen of *Salmonella* infected BALB/c mice[50,51]. This was also supported by the findings of Chakraborty and Roy[38].

Discussion

Apitherapy is an alternative line of treatment based on the use of products of honey bee origin. The term was initially limited to the applications of bee venom

for therapeutic purposes. Lately with the accumulation of information regarding the potential of almost all bee products including pollen, propolis, honey, royal jelly, bee wax and of course bee venom in curative traditional remedies, the term 'Apitherapy' has come to encompass alternative therapeutics based on the entire range of bee products.

It was in view of this background information the present study originated, where *S. aureus* induced oxidative stress in BALB/c mice leads activation of the defense mechanisms in the form of endogenous antioxidants molecules (reduced glutathione) and antioxidant enzymes (GP, GR, GST, CAT, and SOD). The present study showed a statistical significant decrease in the activities of antioxidant enzyme like GP, GR, GST, CAT, SOD, and also decrease in the levels of reduced glutathione after *S. aureus* infection as compared to control. This decrease in activities of antioxidant enzymes and reduced glutathione is explained by several mechanisms which elucidated negative correlation between increased lipid peroxidation and antioxidant enzymes. Oxidative stress leads to increased lipid peroxidation which is assayed by measuring the end product MDA. This increased lipid peroxidation leads to inactivation of antioxidant enzymes and molecules by cross-linking with MDA, which further cause increased accumulation of free radicals like hydrogen peroxide, hydroxyl radical and superoxide, which further enhances more lipids peroxidation. The decrease in antioxidant status might be due to rapid consumption and exhaustion of these antioxidant enzymes and molecules in fighting free radicals generated during lipid peroxidation. This negative correlation between lipid peroxidation and antioxidant defence system is also confirmed and supported from previous studies[52-54]. The antioxidant enzymes and molecules are correlated in their functioning; such as reduced glutathione levels are dependent upon glutathione reductase activity, which is required for conversion of oxidized glutathione to more stable reduced glutathione The antioxidant enzyme, SOD catalyzes dismutation of superoxide anions to hydrogen peroxide and oxygen molecules, hydrogen peroxide free radicals are further converted to water and oxygen molecules by catalase and glutathione peroxidase. Higher oxidative stress leads more oxygen consumption and hence catalase activity, as corroborated from previous studies[55,56].

However, these defence molecules and enzymes are not sufficient to control the burden of oxidative stress and its associated damage to macromolecules therefore, in this direction many plant and animal origin phytochemicals have been found to play important role as potential antioxidants and antimicrobials. Propolis is one such honey bee product, which along with antibiotics ameliorate *S. aureus* mediated reduction in antioxidant molecules and enzymes. Propolis used in the present studies might be inhibiting the penetration of *Staphylococcus* into phagocytic cells and thus, eliminates its proliferation. Propolis along with ampicillin and amoxicillin showed a synergistic behaviour though the mechanism behind this synergism is not known yet. There are some assumptions about it i.e., this combination leads to the formation of a complex which might be lysing bacterial cell wall, or might be interfering with its cell membrane synthesis and hence, directly or indirectly causing death of the bacteria.

Present study also revealed that *S. aureus* infection caused a significant change in the cellular architecture of spleen as revealed by the histopathological changes. Propolis administration showed remarkable curative activity in the spleen of *S. aureus* infected mice as also supported from earlier studies[51,57,58]. Propolis when used along with antibiotics showed synergistic activity as also revealed from previous studies[50,51] and protected spleen from derangements caused by the bacterial infection. The observed histological investigations on spleen tissue of propolis treated and untreated mice further supported the antioxidative potential of propolis. In addition, our studies also proved that ethanolic extract of propolis reduced oxidative damage by decreasing MDA and increasing the status of antioxidant enzymes (GPx, GR, GST, CAT, and SOD) and molecules like reduced glutathione.

Conclusion

The *S. aureus* mediated increased production of reactive oxygen species led to increased lipid peroxidation and reduced glutathione as well as decreased antioxidant status. Treatment with propolis and antibiotics showed curative effect and protected spleen tissue from such infections by decreasing free radical generation, lipid damage, and also by increasing the antioxidant status. The remarkable curative activity observed in spleen also supported the antioxidative potential of propolis. The observed results of oxidative stress parameters and tissue damage are encouraging and validate the use of

propolis alone as well as in combination with standard antibiotics for fighting microbial infections. Further, the bioactive components of propolis responsible for this curative approach should be isolated and the mechanism of action should also be evaluated.

Conflict of interest

The authors declare that there is no conflict of interest.

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