Evaluation the Efficacy of Egyptian Propolis Against Parapox Viruses by Production of IFN-\(\gamma\), TNF-\(\alpha\) and Immunoglobulin in Experimental Rat

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Abstract: Propolis is a natural product extensively used in food and beverages to improve health and to prevent diseases, showing immunomodulatory properties. The goal of the present study was to evaluate the efficacy of Egyptian propolis (ethanolic and water extracts) against parapox viruses. Twenty-five Albino rats, 250-300 g body weight, were randomly divided into five equal groups. G1 was injected with phosphate buffer saline (PBS) mixed with propolis extract (dose 50 mg /kbw) and kept as control. G2 (control positive rats) were inoculated with PPV isolated field virus. Rats of G3 were inoculated PPV mixed with water propolis extract one dose only. G4 was injected PPV mixed with ethanol propolis extract one dose only. G5 inoculated PPV mixed with ethanol propolis extract two dose two weeks apart. The route of inoculation was subcutaneous (SC) and intradermal (ID) for each rat in all groups. Results revealed that Effect of 5- 20% propolis extract concentration on Embryonated Chicken Egg (ECE) and rats found safe, no effect in the nature and color of embryo fluid comparing with control. Water and ethanol propolis extract caused reduction of parapox viruses infectivity titer from \(10^{-3}\) to \(10^{-2}\) and \(10^{-3}\) to \(10^{-2}\) for reference and isolated parapox viruses respectively. The group inoculated with propolis without virus showed slightly suppressed cytokines (TNF-\(\alpha\) and IFN-\(\gamma\)) in the rat sera when comparing to control negative rats. Whereas the cytokine production was strongly stimulate significant increase \((P< 0.05)\) in sera of rat receiving propolis together with PPV. The highest production of IFN-\(\gamma\) and TNF-\(\alpha\)(0.536±0.095, 0.406±0.079 and 0.341±0.052) and (0.295 ± 0.065, 0.253 ± 0.058 and 0.267 ± 0.055) in G5, G4 and G3 measured by ELISA (Means ODs ± SD), respectively. Control positive infected rats with parapox viruses without propolis showed signs of the inflammation and swelling at the site of injection after the 1st week post infection and on necropsy showed congested with petichial hemorrhage in liver, spleen and kidney. Histopathological studies showed acute necrotic hepatitis accompanied with disseminated intravascular coagulopathy (DIC), which is a pathognomonic process. The present study proved that the propolis extract have many effects on the TNF-\(\alpha\) and IFN-\(\gamma\) cytokines and humoral immune response may be exploited for the development of effective natural antiviral and immunostimulant product uses in human and animals.

Key words: Parapox virus • Orf • PPV • ELISA • Propolis • IFN-\(\gamma\) • TNF-\(\alpha\)

INTRODUCTION

Animal infectious diseases, especially the viral diseases, are worldwide concerned as they usually cause a great loss in domestic animal [1]. Because virus has unique biological characteristics and pathogenesis, there are no effective treatment methods for viral diseases [2]. A few chemical drugs have been used, but their clinical effects are not satisfied and there are obvious negative effects such as drug residues, drug tolerance, high recurrence rate, environmental pollution and so on [3]. Therefore it becomes so urgent to study and develop new-type of natural antiviral products with high efficiency and low toxicity in the treatment of human and animals viral diseases [4]. One such product is propolis [5]. The chemical composition of propolis is complex and has not been completely elucidated. In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10%
essential and aromatic oils, 5% pollen and 5% of various other substances, including organic debris [6]. The medicinal use of bee products was also prominent in Islamic history [7]. So far propolis has been reported to have many different biological and pharmacological properties of different preparations have been reported as antimicrobial, antifungal, antiinflammatory, antitumor and antiapoptotic properties [7-9]. A number of authors have reported that propolis extracts have an inhibitory effect on the development of an infection process caused by viruses affecting animals and humans [10-13]. Although the immunostimulator and immunomodulator activities have been demonstrated [14-17]. Parapoxvirus is a highly contagious, zoonotic, viral skin disease that affects domesticated and wild ruminants as well as human where it represent an occupational hazard among people who handle with infected animals [18, 19]. Parapoxvirus was isolated and identified as a member of the genus parapoxviruses in the family Poxviridae from man and animals in worldwide and Egypt [20, 21]. There are numerous confirmed studies that propolis is able to activate immune system in mouse and human as follows; increasing IL1, IL2, IL4 and antibody response [22]. Propolis is non-toxic, it did not induce any alterations in their weight [15]. The present study aimed to evaluate the efficacy of propolis on production of tumor necrosis factor-alpha (TNF-α) and interferon gamma (IFN-γ) cytokines levels and antibody titer were measured by ELISA to characterize a potential immunomodulatory effect.

**MATERIALS AND METHODS**

This study was carried out according to guidelines for animal experimentation and approved by the Institutional Animal Care and Use Committee, National Research Center Animal Care Unit, Dokki, Giza, Egypt.

**Animal Used:** Twenty five clinically healthy Albino rats (250-300 g body weight) were obtained from Animal house of National Research Center Cairo Egypt and randomly divided into 5 groups for experiment. The Animals were housed in a well-ventilated animal room under standardized conditions of 24°C; relative humidity 50±5% and 12 hours light/dark cycle at the Animal House, National Research Center, Giza, Egypt. Feed and water were supplied ad libitum to meet the requirements of the NRC [23]. Rats were acclimatized for 15 days before the start of the experiment.

**Experimental Design:** This experiment was carried out at the Experimental Animal Rat Unit of Lab Animal House, National Research Center, Dokki, Giza, Egypt. Rats were randomly divided into five equal groups, of 5 rats each. then housed under strict hygienic and isolation conditions, as follows: Group 1 (G1) was injected subcutaneous (SC) and interadermal (ID) with phosphate buffer saline (PBS) mixed with propolis extract (dose 50 mg/ml for one time), G2 control positive rats inoculated with PPV isolated field virus (SC/ID), G3 were inoculated PPV mixed with water propolis extract (SC/ID) one dose only, G4 was injected PPV mixed with ethanol propolis extract (SC/ID) one dose only and G5 inoculated PPV mixed with ethanol propolis extract (SC/ID) two dose two weeks apart. (strict isolation measures were taken to avoid any spread of the infection) Daily observation of the experimental animals was recorded. Blood samples were collected by retro orbital venous plexus puncture from all groups at 0, 7, 14, 28, 35 and 42 days of experiment in sterile plain centrifuge tubes and allowed to clot at 37°C for 1 hour. Serum was separated by centrifugation for determination of cytokines (TNF-α and IFN-γ) and anti PPV antibody by Enzyme-linked immunosorbent assay (ELISA). At the end of experiment (at the 42nd day post infection), rats of all groups were then euthanized and specimens from liver and kidney and spleen were taken for histopathological examinations.

**Extraction of Propolis**

**Ethanol Extraction of Propolis (EEP):** Propolis was collected from beehives located in Egypt. In the first step propolis was extracted by ethanol, propolis was ground, macerated with four–five times (w/v) absolute ethanol (95–96%) for 1–10 days and agitated for 10 min every day. The ethanol was evaporated and the dry material is re-dissolved in phosphate buffer solution (pH 6.2). The mixture was centrifuged at 7000 rpm for 15 min at 25°C. The supernatant was collected and the pellet was re-extracted with 100 ml ethanol. After pooling the supernatants of both steps, a final concentration of 50 mg/ml (pH 7.2) were prepared according to Nolkemper et al. [24].

**Water Extracts of Propolis (WEP):** About 200 ml of ethanol extracts of propolis from pervious step was poured into 500-ml flask and kept on a magnetic stirrer. 200 ml of 20 mM phosphate buffer was added to the ethanol extract of propolis and was mixed for 20 min at 25°C. The mixture was centrifuged at 7000 rpm for 15 min
and the supernatant was collected. Water-soluble compounds remained in the aqueous phase which was a solution with a light yellow color. The lower part was dark brown and very sticky. The water extracts of propolis were concentrated by freeze-drying method and used for the next step according to Türkez et al. [25].

**Sterilization of Propolis Extract:** The water and ethanolic extracts of propolis (WEP and EEP) was sterilized by 0.22 im Millipore syringe filter and then diluted to 50 mg/ml of final concentration in (PBS) solution (pH 7.2) and used at dose of 50 mg/kg of body weight.

**Toxicity of propolis:**

**Toxicity of Propolis to Embryonated Chicken Eggs (ECE):** The toxicity of propolis to specific pathogen free (SPF) in ECE was examined as follows: incubated 0.2 ml of 5 – 20% propolis extract into chorio-allantoic membranes (CAM) and chorio-allantoic sac (CAS) and incubated at 37°C for 7 days. Each day the SPF was examined. The maximum tolerated dose was taken as the maximum non-toxic concentration.

**Toxicity of Propolis in vivo:** Five rats were injected SC/IP (intra-peritoneal) with 1 ml of 5 – 20% propolis extract twice dose interval two weeks. Behavioral alterations, inflammatory effects, illness and weight changes were recorded for 4 weeks post-treatment. Control animals were injected with PBS.

**Virus Propagation:** The PPV was propagated in CAM of SPF. Stock viruses were prepared as previously described by Zeedan et al. [20]. The egg-adapted Parapoxvirus was provided by Dr. G.S. Zeedan, NRC, Cairo, Egypt. Virus was propagated in the CAM of SPF embryonated chicken eggs (11 days age). Both CAM and AF were harvested and ground in 0.1M sterile PBS containing antibiotics (penicillin 100 IU/ml, streptomycin 100 ig/ml and kanamycin 50 µg/ml). The homogenate was frozen at -20°C and thawed three times and then centrifuged at 3000 rpm for 15 min. Then supernatant was titrated and stored at-80°C until used.

**Determination of antiviral activity by Pock Reduction Assay:** Virus titers were estimated from cyto pathogen city by tenfold dilution method; 0.02 ml of each dilution was inoculated into the five CAM of ECE and expressed as 50% egg infectious dose per ml (EID50/ml). Calculated for each tested propolis was performed according to Reed and Munch [26].

Enzyme Link Immunosobant Assays (ELISA): ELISA was prepared by the reagent kits method concentrations of TNF- α, IFN- γ (supplied from Diaclone A Tepnel Co., 25020 Besancon Cedex – France) and anti PPV antibody were determined by using ELISA, briefly, a 96-well flat bottom was coated with capture antibody specific to each cytokine. The plate was washed and blocked before 100 ml of the rats sera and serially diluted specific standards were added to the respective wells. Following a series of washing, the captured cytokine was detected using the specific conjugated detection antibody. The chromogen/substrate reagent was added into each well and after color development, the plate was read at 450 nm using an ELISA plate reader.

**Pathological Studies:** Tissue specimens were collected immediately from liver, kidney and spleen after euthanized of animal groups at the end of experiment then fixed in 10% formal saline. Dehydrated, cleared and embedded in paraffin blocks were done. Paraaffin sections of 5µ thickness were prepared, stained by H&E and examined microscopically for detection of histopathological alterations [27].

**Statistical Analysis:** All data were subjected to statistical analysis including the calculation of the mean and standard Deviation. Significance between data of control and infected groups was evaluated by the Student t-test at P < 0.05 according to Petrie and Watson [28] using SPSS for windows version 15 computer program.

**RESULTS**

**Extraction of Propolis:** The ethanol extract of propolis was collected and consisted of near 35% propolis. The extract had a clear reddish color. Additions of buffer to the ethanol extract of propolis caused sedimentation of the low water-soluble materials, which are very sticky. After centrifugation of the mixture, the liquid phase was separated and sterilized consisted of near 7% of dry propolis and then sediment was weighted to be 28–30%.

**Antiviral Activity:** Data presented in Table (1): revealed that both viruses strains isolated and reference viruses were decreased nearly 2-3 log infectivity titer means all viruses susceptible to effect propolis extract.

**Immunological Studies**

**Humeral Immune Response:** The TNF-α, IFN-γ and antibody against PPV levels in the serum of rats are
Table 1: Effect of propolis on PPV activity tested pock reduction assay (Means±SD)

<table>
<thead>
<tr>
<th>Log infectivity titer EID50</th>
<th>PPV without propolis</th>
<th>PPV mixed with ethanol propolis extract</th>
<th>PPV mixed with water propolis extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reference PPV</strong></td>
<td>5.3±0.130</td>
<td>2.9±0.218</td>
<td>2.5±0.144</td>
</tr>
<tr>
<td><strong>PPV isolated strain</strong></td>
<td>4.64±0.226</td>
<td>2.3±0.114</td>
<td>2.8±0.123</td>
</tr>
</tbody>
</table>

Table 2: Effect of propolis extracts against PPV on production of IFN-γ in rat sera measured by ELISA

<table>
<thead>
<tr>
<th>Groups</th>
<th>3D</th>
<th>7D</th>
<th>14D</th>
<th>28D</th>
<th>35D</th>
<th>42D</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.145±0.18</td>
<td>0.148±0.24</td>
<td>0.155±0.08</td>
<td>0.148±0.015</td>
<td>0.264±0.012</td>
<td>0.155±0.05</td>
<td>0.168±0.045</td>
</tr>
<tr>
<td>G2</td>
<td>0.336±0.01</td>
<td>0.285±0.032</td>
<td>0.170±0.072</td>
<td>0.255±0.019</td>
<td>0.180±0.07</td>
<td>0.195±0.015</td>
<td>0.231±0.061</td>
</tr>
<tr>
<td>G3</td>
<td>0.563±0.04</td>
<td>0.654±0.069</td>
<td>0.356±0.065</td>
<td>0.315±0.10</td>
<td>0.295±0.06</td>
<td>0.325±0.061</td>
<td>0.341±0.052</td>
</tr>
<tr>
<td>G4</td>
<td>0.396±0.31</td>
<td>0.354±0.17</td>
<td>0.452±0.55</td>
<td>0.445±0.17</td>
<td>0.325±0.16</td>
<td>0.435±0.014</td>
<td>0.406±0.079</td>
</tr>
<tr>
<td>G5</td>
<td>0.455±0.48</td>
<td>0.460±0.16</td>
<td>0.505±0.093</td>
<td>0.695±0.11</td>
<td>0.622±0.205</td>
<td>0.489±0.16</td>
<td>0.536±0.095</td>
</tr>
</tbody>
</table>

Table 3: TNF-α production by stimulated with propolis extract in rats sera tested by ELISA

<table>
<thead>
<tr>
<th>Groups</th>
<th>3D</th>
<th>7D</th>
<th>14D</th>
<th>28D</th>
<th>35D</th>
<th>42D</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.115±0.013</td>
<td>0.145±0.030</td>
<td>0.155±0.018</td>
<td>0.132±0.007</td>
<td>0.164±0.016</td>
<td>0.125±0.017</td>
<td>0.139±0.018</td>
</tr>
<tr>
<td>G2</td>
<td>0.239±0.141</td>
<td>0.315±0.040</td>
<td>0.285±0.017</td>
<td>0.175±0.022</td>
<td>0.180±0.024</td>
<td>0.195±0.023</td>
<td>0.231±0.048</td>
</tr>
<tr>
<td>G3</td>
<td>0.251±0.005</td>
<td>0.224±0.015</td>
<td>0.356±0.040</td>
<td>0.315±0.056</td>
<td>0.235±0.021</td>
<td>0.225±0.025</td>
<td>0.267±0.055</td>
</tr>
<tr>
<td>G4</td>
<td>0.296±0.054</td>
<td>0.264±0.0098</td>
<td>0.252±0.037</td>
<td>0.245±0.043</td>
<td>0.225±0.062</td>
<td>0.235±0.0448</td>
<td>0.253±0.058</td>
</tr>
<tr>
<td>G5</td>
<td>0.355±0.045</td>
<td>0.360±0.012</td>
<td>0.305±0.04</td>
<td>0.312±0.086</td>
<td>0.212±0.089</td>
<td>0.225±0.054</td>
<td>0.295±0.065</td>
</tr>
</tbody>
</table>

Table 4: Determination of propolis extract effect on the production of anti PPV IgG tested by ELISA

<table>
<thead>
<tr>
<th>Groups</th>
<th>3D</th>
<th>7D</th>
<th>14D</th>
<th>28D</th>
<th>35D</th>
<th>42D</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.153±0.0148</td>
<td>0.261±0.037</td>
<td>0.216±0.025</td>
<td>0.252±0.016</td>
<td>0.258±0.027</td>
<td>0.181±0.015</td>
<td>0.194±0.049</td>
</tr>
<tr>
<td>G2</td>
<td>0.175±0.0087</td>
<td>0.195±0.048</td>
<td>0.185±0.022</td>
<td>0.252±0.0145</td>
<td>0.268±0.016</td>
<td>0.182±0.010</td>
<td>0.207±0.039</td>
</tr>
<tr>
<td>G3</td>
<td>0.248±0.015</td>
<td>0.562±0.019</td>
<td>0.589±0.058</td>
<td>0.654±0.072</td>
<td>0.522±0.051</td>
<td>0.526±0.038</td>
<td>0.490±0.155</td>
</tr>
<tr>
<td>G4</td>
<td>0.324±0.036</td>
<td>0.654±0.067</td>
<td>0.752±0.08</td>
<td>0.815±0.095</td>
<td>0.825±0.107</td>
<td>0.745±0.103</td>
<td>0.650±0.175</td>
</tr>
<tr>
<td>G5</td>
<td>0.356±0.045</td>
<td>0.724±0.102</td>
<td>0.856±0.104</td>
<td>0.922±0.115</td>
<td>0.956±0.105</td>
<td>0.854±0.057</td>
<td>0.741±0.195</td>
</tr>
</tbody>
</table>

expressed as the titer, which has higher optical density (OD) than reference value, based on the mean value of negative sera±2SD.

Data presented in Table (2) IFN-γ production stimulated with propolis extracts on rats infected with PPV (G3) rats inoculated PPV mixed with water propolis extract, rats of G4 inoculated PPV mixed with ethanol propolis extract one dose SC/ID and G5 injected PPV mixed with ethanol propolis extract SC/ID two dose two weeks apart compared with rats of G2 rats inoculated by PPV (control positive group) and G1 (control negative group).

Table (3) showed G1: rats received PBS mixed with propolis injected by (SC/ID) control negative group, G2: control positive rats inoculated by PPV isolated field virus (SC/ID), G3: rats received PPV mixed with water propolis extract one dose (SC/ID) only, G4: rats received PPV mixed with ethanol propolis extract one dose (SC/ID) only and G5: rats received PPV mixed with ethanol propolis extract (SC/ID) two dose two weeks apart. TNF-α production by rats stimulated with propolis extracts mixed with PPV on rats groups.

Table (4): showed that Rat IgG production stimulated by propolis extracts on rats infected with PPV. G1 received PBS mixed with propolis (SC/ID) control negative group, G2 control positive rats inoculated by PPV isolated field virus (SC/ID), G3 received PPV mixed with water propolis extract one dose (SC/ID) only, G4 received PPV mixed with ethanol propolis extract one dose (SC/ID) only and G5 received PPV mixed with ethanol propolis extract (SC/ID) two dose two weeks apart.

Postmortem and Histopathology Findings: After forty two days all rats were euthanized, Postmortem and histopathology investigation were performed on target
Propolis reveals a broad spectrum of biological activities and is used in food and folk medicine with renewed interest in its antimicrobial and antiviral potential [32]. It is well understood that bees collect propolis to seal their hive and to prevent the decomposition of creatures which have been killed by bees after an invasion of the hive. Therapeutic application of propolis against viral and bacterial infections has been described previously by Lederman et al. [33]. This study focused on efficacy of propolis extract as antiviral activity by cytokine and antibody production.

In the present study the concentration 5-20% propolis extract was observed safe, no effect in the nature and color of embryo fluid comparing with control as well as experimental animals in agreement with Yunpeng et al. [34].

The effect of water and ethanolic propolis extract on PPV activity was determined by pock reduction assays EID50. WEP and EEP were showed reduction infectivity titer from $10^{-5.5}$ to $10^{-7.5}$ and $10^{-6.5}$ to $10^{-3.9}$ for reference and isolated PPV viruses. Antiviral effect of propolis extract may be blocked the viral infection by blocking the cell membrane receptor for PPV or induce internal changes in the host cells, which in turn affect the virus replication cycle or due to production alpha interferon from CAM of SPF which blocked viral infection other health cells as in Table (1) and Fig (1). This result was in agreement with Schnitzler et al. [35]. who recorded that the propolis extract caused sharp reduction in small pox viruses infectivity titer. They observed that the concentration of viruses were reduce $10^{-5}$ to $10^{-4}$ and they infectivity titer were reduced 21 and 25 time than control group. Whenever propolis was added to PPV before injection to CAM, the plaque size and number was greatly reduced, this finding was agreed with Schnitzler et al. [35].

Our results suggested that both propolis extracts may be interfere with virion envelope structures or are masking viral compounds which are necessary for adsorption or

DISCUSSION

Contagious ecthyma is a highly contagious viral disease of sheep, goats, cattle and occasionally humans with worldwide distribution. It is caused by filterable virus which is the type species of the parapoxviruses genus in the family Poxviridae [29]. There are several possible reasons why orf virus can repeatedly reinfect its host. First, the infection is acute and apparently restricted to epidermal keratinocytes in vivo. This may allow the virus time to replicate prior to the recruitment of a critical level of antiviral immunity. Second, virus infection may not stimulate an appropriate protective response. Finally, the virus may have evolved mechanisms to subvert or interfere with components of a protective immune response as has been demonstrated with other poxviruses. For example, vaccinia virus is able to block the anti-viral effects of interferon by producing an interferon-binding protein and an inhibitor of an interferon-induced. A dramatic increase in the prevalence of PPV infection was observed by Guo et al. [30]. Milker’s nodules continue to be a public health problem in developing countries [12] because of its contagious nature and economic importance of the PPV. Isolation of PPV in Egypt was done by Zeedan et al. [20] and Shemies [31].

![Fig. 1: Log infectivity titer of viruses treated with propolis extract tested of pock reduction assay (means±SD)](image)

Image caption: Fig. 1: Log infectivity titer of viruses treated with propolis extract tested of pock reduction assay (means±SD)
entry into host cells. Recently it has been reported that propolis demonstrated a similar antiviral effect. On the other hand disagreed with results found by Amoros et al. [36] investigated that in vitro antiviral activity of resin balsam against HSV-1 and could detected a virucidal effect when HSV-1 was pretreated with propolis did not inhibit viral replication.

Propolis extract effect on PPV activity by significantly increase IFN-γ titers in the rat sera of G3, G4 and G5 compared with control negative group (G1) which received PBS mixed with propolis inoculated by SC and ID. Rats of G1 did not have any change. The highest production of IFN-γ (0.536±0.095, 0.406±0.079 and 0.341±0.052) was recorded in serum of G5, G4 and G3 measured by ELISA (Means ODs±SD), respectively. It is possible that propolis have an immunological activity contributing to the limitation of PPV growth by elevating IFN-γ production from infected rats. However, propolis without virus was failed to directly production of IFN-γ.

Thus, propolis is active and augmenting IFN-γ production in infected rats or immunological activity between 3-42 days, these finding was in agreement with Marquez et al. [37]. It seems that the effect of propolis in production of this cytokine levels may be increased. The ethanolic propolis extract stimulated TNF-α levels in rats treated with 50 mg dose for 1st and 2nd weeks when comparing to the same group before treatment. The ELISA- TNF-α levels in G2, G3, G4 and G5 slightly increased in all groups but still more than G1 control negative group. It seems that the effect of propolis in producing cytokines is dependent on the dose and duration of propolis administration. It is suggested that inoculated propolis extract only suppresses TNF-α production in low dose, but with increasing of dose, the levels of this cytokine may be increased. As illustrated in Fig. (3), PPV plus propolis G3 as well as in G5 was significantly higher than other groups (P < 0.05).
Fig. 5: Histopathology changes in the liver, kidney and spleen of rats, (A): Liver of animals sacrificed at the 42nd day post infection showed vacuolar degeneration was the most common pathological alteration and focal leukocytic infiltration and inclusion bodies were obvious in kupffer cells in most cases compared to the infected group without propolis, (B): Kidney showed degenerative changes in epithelial cells lining renal tubules at the same level of cortex and medulla, pyknosis with coagulation of the cytoplasm of the renal epithelium and (C): Spleen of animal was enlarged, hyperplasia of the lymphoid follicles and widening of sinusoids (H&E X400).

The anti PPV antibodies titer produced in G3, G4 and G5 increase antibody levels from the 7th to the 42nd days this finding was agreed with Shaapan et al. [31] and Blonska et al. [38]. They found that antibody titers in groups treated with the antigen and propolis by different routes were higher than that of the antigen group and may be attributed to the ability of propolis for modulating the synthesis of antibodies. The ethanolic and water extract of Egyptian propolis increase the cytokine production when administrated in combination with PPV in rats enhanced specific and nonspecific immune response. In The control positive infected rats with PPV without propolis showed signs of the inflammation and swelling at the site of injection after the 1st week post infection. Examination of control positive rats group infected with PPV strain showed scabs lesions at the inoculated part congested vessels with subcutaneous hemorrhage, presence of necrotic foci in the liver, spleen and congested kidneys. Rats of G1 administrated propolis only showed subcutaneous hyperemic patches and slightly congested liver, kidney and spleen. Rats of the G3, G4 and G5 administrated propolis and PPV appeared normal or mild lesions. Some rats showed multiple subcutaneous scabs in the front of abdominal skin.

In conclusion, the effect of WEP and EEP on PPV infectivity titer showed antiviral effects which reduced infectivity titer to 2-2.5 log for reference and isolated PPV viruses. Our results showed clearly that the ethanolic and water extract of propolis alone caused slightly suppressed TNF-α and IFN-γ cytokines in the rats’ serum when compared to controls negative group. Whereas, the cytokine production was strongly stimulated in rat sera receiving propolis together with PPV increased TNF-α, IFN-γ and IgG production. The present study proved that the propolis extract have many effects on the TNF-α and IFN-γ cytokines and humoral immune response may be exploited for the development of effective natural antiviral and immunostimulant product uses in human and animals.

REFERENCES


