Tsuchiya et al., J Vet Sci Med Diagn 2018, 7:2
DOI: 10.4172/2325-9590.1000252

Journal of Veterinary Science & Medical Diagnosis

Research Article

A SCITECHNOL JOURNAL

Evaluation of Preventive Effects of Brazilian Green Propolis on Equine Herpes Virus 9-Induced Acute Encephalitis

Yuya Tsuchiya[1], Hoda A Abd-Ellatieff[2], Abdelrahman A Abou Rawash[2] and Tokuma Yanai[1]*

Abstract

In this study, we aimed to evaluate the preventive effects of Brazilian green propolis on equine herpes virus (EHV)-induced encephalitis in a hamster model. We first investigated the antiviral activity of Brazilian green propolis against EHV-9, and then, examined the effects of propolis on the kinetics and production of cytokines. Sixty-five male, Syrian hamsters were divided into two treatment groups and control group. The 1st group is a control. The second group was infected intranasally with 50 µl of EHV-9 [3 × 10^4 plaque-forming units (pfu)] without propolis treatment. The third group received propolis at 500 mg/kg of ethanol extract of Brazilian green propolis by a gavage for 7 days, followed by intranasal inoculation with 50 µl of EHV-9 (3 × 10^4 pfu). Pre-treatment with propolis was not effective in preventing EHV-9-induced encephalitis via intranasal route. This speculation is supported by similar clinical signs and similar immunohistochemistry based on viral antigen distribution in the brain of infected hamsters in groups 2 and 3 from 3rd day post inoculation (dpi). There were significant increases in cerebral mRNA levels of IL-2, IL-10, and IFN-γ after propolis administration in the propolis-treated group before and after EHV-9 challenge compared with the control non-treated group. In conclusion, Brazilian green propolis showed no obvious effect on the prevention of acute EHV-9-induced acute encephalitis in a hamster model, despite its immune-enhancing activity.

Keywords

Cytokine; EHV-9; Encephalitis; Pathogenesis; Propolis

Introduction

Propolis has been widely used in folk medicine for many years as pharmaceuticals or dietary supplements for the prevention or treatment of inflammatory diseases [1]. There are numerous studies on the pharmacological properties and biological activities of propolis, including anti-inflammatory, anti-bacterial, anti-fungal, anti-oxidant, anti-cancer, and antiviral effects [2,3]. Propolis (bee glue) is a resinous substance collected by honey bees from various plant sources. It is used to seal holes in honeycombs, smooth out the internal walls, and protect the entrance to intruders. Propolis is a bee product that comprises a mixture of polyphenols, flavonoid aglycones, phenolic acids and their esters, and phenolic aldehydes and ketones [4]. It is rich in biochemical constituents, mainly phenolic acid components, which play a role in antiviral activity. It has been reported that constituents such as caffeic acid, p-coumaric acid, benzoic acid, galangin, pinocembrin, and chrysin may be effective against herpes simplex virus in cell culture [5,6]. Propolis and its derivatives have the ability to inhibit virus propagation via reduction of viral multiplication and virucidal action. Several studies have shown the effect of propolis on the RNA and DNA of different viruses *in vitro*, including herpes simplex type 1, herpes simplex type 2, adenovirus type 2, poliovirus type 2, vesicular stomatitis virus [7], vaccinia virus, and the virus responsible for Newcastle disease [8]. Propolis has been used as a potential immune regulator [9,10]. Recently, it was reported that Brazilian green propolis has immune enhancing action both *in vitro* and *in vivo* [11,12].

Equine herpesvirus-9 (EHV-9), a varicellovirus belonging to the subfamily Alpha-herpes virinae, is the newest member of EHV and is closely related to EHV-1. EHV-9 was first described in an outbreak of disease in Thomson's gazelles (Gazellathomsonii) in a Japanese zoo [13,14].

Emerging EHV-9 infection in domestic animals is also a concern, as various domestic animals can be easily infected via nasal route [15-18]. The pathogenesis of EHV-9 following nasal infection has been studied in a suckling hamster model [15]. EHV-9 propagated in the olfactory epithelium within few hours (12-24/hrs) gained access to the brain through the olfactory and trigeminal nerves [14,15].

In the present study, we investigated the preventive effects of Brazilian green propolis on EHV-9-induced acute encephalitis as well as its immune activation functions by measuring cytokine levels.

Material and Methods

Animals

Sixty five, 4-week-old male, specific pathogen-free Syrian hamsters (*Mesocricetus auratus*) were used in this study. The animals were purchased from an experimental animal breeder (SLC Inc., Hamamatsu, Japan). All animal experiments were performed according to the code of ethics of Research and Animal Resources Committee, Faculty of Applied biological science, Gifu University, Japan, approved by Gifu University Animal Experiment Committee. The animals were kept in a well-ventilated room at 22-24°C and 50% relative humidity, with 12 hr/12 hr light/dark cycle. Food and water were provided ad libitum. After acclimation for 5 days, they were randomly divided into three groups: (Group 1) control non-treated group (20 animals), (Group 2) EHV-9 inoculated without propolis treatment group [20 animals), and (Group 3) propolis treated and EHV-9 inoculated group (25 animals).

Virus seed

Original seed stocks of EHV-9 [P19, 5th 102 passages in Madine-Derby bovine kidney cells (MDBK)] are the source of inocula of virus infection used in this experiment. The viruses were titrated by plaque assay in MDBK cells, which were used for virus propagation.

Experimental design

Group 1 and group 2 were intranasally inoculated with 50 µl (MEM–α) divided between both nostrils for 7 days. Group 3 received

***Corresponding author:** Tokuma Yanai, DVM, Ph. D, Laboratory of Veterinary Pathology, Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Japan, E-mail: yanai@gifu-u.ac.jp

Received: March 21, 2018 **Accepted:** April 10, 2018 **Published:** April 15, 2018

propolis as 500 mg/kg of ethanol extract of Brazilian green propolis (obtained from API Co., Ltd. Gifu, Japan) by gavage for 7 days. From 8th day from the start of the experiment, Group 2 and group 3 were infected intranasally with 50 µl of EHV-9 [3×10^4 plaque forming units (pfu)]. Each group was housed separately to prevent cross-infection. The experimental animals were kept under observation daily until the end of the experiment for appearance of any clinical signs post viral infection. Five hamsters from group 3 were euthanized before viral challenge by 12 hr. Five hamsters from each of the three groups were euthanized at 2nd, 3rd, 4th and 5th dpi, and the samples/specimens were immediately collected (Table 1).

Necropsy and postmortem examination

Euthanasia was performed by intraperitoneal administration of pentobarbital sodium (100 mg/kg). The brains, heart, lungs, kidneys, liver, and spleen were removed and examined for gross lesions. The brain was divided into two halves. One-half of the brain was collected in sterilized Eppendorf tubes containing RNA for RNA and DNA extraction. The second half of the brain and suitable specimens from other organs were immediately fixed in 10% buffered paraformaldehyde (average period of fixation was 3-4 days at 24°C for routine paraffin section preparation and histopathological and immunohistochemical examination.

RNA extraction for viral and inflammatory cytokine detection

Total RNA extraction from the brain and lung tissues was performed according to the Qiagen All Prep DNA/RNA/Protein Mini kit protocol. Total RNA samples were reverse transcribed by using Quanti TectR Reverse Transcription Kit (Germany). cDNA was then used for EHV-9 detection using its specific primers [19], and interleukin (IL)-2, IL-10, and interferon gamma (IFN-γ) using hamster-specific primers [20,21].

Real-time PCR and amplification protocol

PCR amplification and analysis were performed using Takara Thermal Cycler. Dice Real Time System with version 4.0 software. All reactions were performed with the Thermal Cycler Dice single Dice. In this study, DNA master SYBR green I kit was used [Takara Clontech, SYBR^R premix Ex Taq^{TM} (TliRNaseH plus)] at a volume of 25 µl in each reaction capillary. For quantification of cytokines, 5 µl of cDNA was added before the capillaries were capped, centrifuged, and placed in Light Cycler sample carousel. The amplification conditions of the target cDNA was for 40 cycles (95°C for 30 sec, 95°C for 10 sec, and 60°C for 10 sec, and a variable extension time at 72°C for10 sec). Dissociation curve analysis was performed immediately after amplification with continuous fluorescence acquisition.

Histopathological and immune histochemical examination

The brain, heart, lungs, liver, spleen, and kidneys were collected immediately after necropsy and fixed in 10% buffered paraformaldehyde. After fixation, the organs and tissues were dehydrated and embedded in paraffin wax, sectioned into 3-µm sections, stained with hematoxylin and eosin (HE), and examined under a light microscope. Paraffin-embedded sections (4 µm) of the brain were immune labeled with EHV-9 rabbit antiserum using the En Vision+ System HRP-labeled polymer (Dako). The primary antibody was EHV-9 antiserum used at a dilution of 1:800. Subsequently, a secondary antibody (biotinylated anti-rabbit IgG, DAKO Cytomation, USA) was applied, with Liquid Diaminobenzidine (DAB) Substrate Chromogen System (DAKO Cytomation) used as the chromogen and hematoxylin as the counterstain, as described previously [7,22]. Known tissue sections from EHV-9-infected hamsters were used as positive control samples, and sera from non-immunized rabbit and goat were used as a negative control.

Statistical analysis

Two-way analysis of variance (ANOVA) test using the Graph Pad Prism 5 software were used for statistical analysis of changes in cytokines levels of (IL-2, IL-10, and INF-γ) genes from brain tissues. Comparisons were considered significant at a P value ≤ 0.05.

Results

Clinical findings and death

All animals in the EHV-9-inoculated groups (the 2nd and 3rd groups) showed similar clinical signs on the 3rd dpi. The early clinical signs included depression, salivation, nasal discharge, lack of coordination, and periodic convulsions, which then developed into lateral recumbency and coma in most of the animals. There was no dead animal on the 3rd dpi, two dead animals in both groups on the 4th dpi, and three dead animals on the 5th dpiin the second group. In addition, four dead animals were observed on the 5th dpi in the third group. No deaths or clinical signs were observed in the non-treated control group. The number of affected animals remaining on each day of experiment is shown in Table 2.

Changes in bodyweight gain

Both the EHV-9-inoculated groups with or without propolis treatment showed a significant reduction in bodyweight gain on the 5th dpi compared to the control group, while the control group showed a normal pattern of bodyweight gain.

Gross findings

No specific gross abnormalities were observed in the organs and tissues at necropsy, except congestion of the meningeal and cerebral blood vessels.

Table 1: Experimental design for the groups of hamsters.

Groups	Dose/Route	Nos. of animals euthanized on DPI					
		12hr before challenge	2nd	3rd	4th	5th	Total
Group 1(Control)	50 *µl* MEM–α intranasal	-	5	5	5	5	20
Group 2 (EHV-9 infected without propolis treatment)	50 *µl* (1 × 104 PFU) Intranasal	-	5	5	5	5	20
Group 3 (propolis treated group with EHV-9 challenge)	500 mg/kg of Ethanol extract of Brazilian green propolis for 7 days then challenged by50 *µl* (1 × 104 PFU) Intranasal	5	5	5	5	5	25

Histopathological examination

In the EHV-9-inoculated groups, there were various degrees of non-suppurative encephalitis characterized by perivascular cuffing, focal or diffuse gliosis, neuronal degeneration and neuronal loss, and frequent eosinophilic intranuclear inclusion bodies in the degenerating neuronal cells in infected animals (Figure 1A, 1B, 1C). Lesions were predominant in the olfactory bulb and gray matter of the cerebrum in the brains from both theEHV-9-inoculated groups. There was no significant difference between the second group and third group in the prevalence of neuronal lesions. No lesions were observed in the brains from the non-treated control group.

Viral antigens by immunohistochemistry

Viral antigens were detected in the olfactory bulb from the 3rd dpi. The amount of viral antigens increased on the 4th dpi in the second group and was more abundant on the 5th dpi in the third group (Figure 1D). No positive immunostaining was observed in the olfactory bulb on the 2nd dpi or in the control group.

Detection of viral DNA and cytokines levels using qRT-PCR

Viral DNA was detected in the brain tissue of infected animals from the 2nd dpi until the end of the experiment (5th dpi).

In this study, administration of 5000 ppm of ethanol extract of Brazilian green propolis by gavage for 7 days resulted in a 19-15, 9, fold increase in mRNA levels of IL-2, IL-10, and IFN-γ cytokines respectively in propolis treated hamsters at the 7th day from propolis administration in comparison to the control non-treated group.

There were also a marked increase in mRNA levels of IL-2, IL-10, and IFN-γ in the brain tissue of all EHV-9-infected groups either propolis treated (3rd group) (Figure 2A, 2B, 2C, 2D) or untreated group (2nd group) (Data under publication). No significant differences were observed between the 2nd and the 3rd groups in cytokines levels.

Table 2: The rate of infection at each day of the experiment.

Groups	0 day (day of infection)		2nd DPI		3rd DPI		4th DPI		5th DPI	
	1st 12 hr	2nd 12 hr	1st 12 hr	2nd 12 hr	1st 12 hr	2nd 12 hr	1st 12 hr	2nd 12 hr	1st 12 hr	2nd 12 hr
1st Control	0/20	0/20	0/20	0/15	0/15	0/15	0/10	0/10	0/5	0/5
2nd EHV-9 infected without propolis treatment	0/20	0/20	0/20	0/15	0/15	0/10	2/10	8/10	4/5	1/1
3rd propolis treated group with EHV-9 challenge	0/20	0/20	0/20	0/15	0/15	0/10	2/10	8/10	3/5	2/2

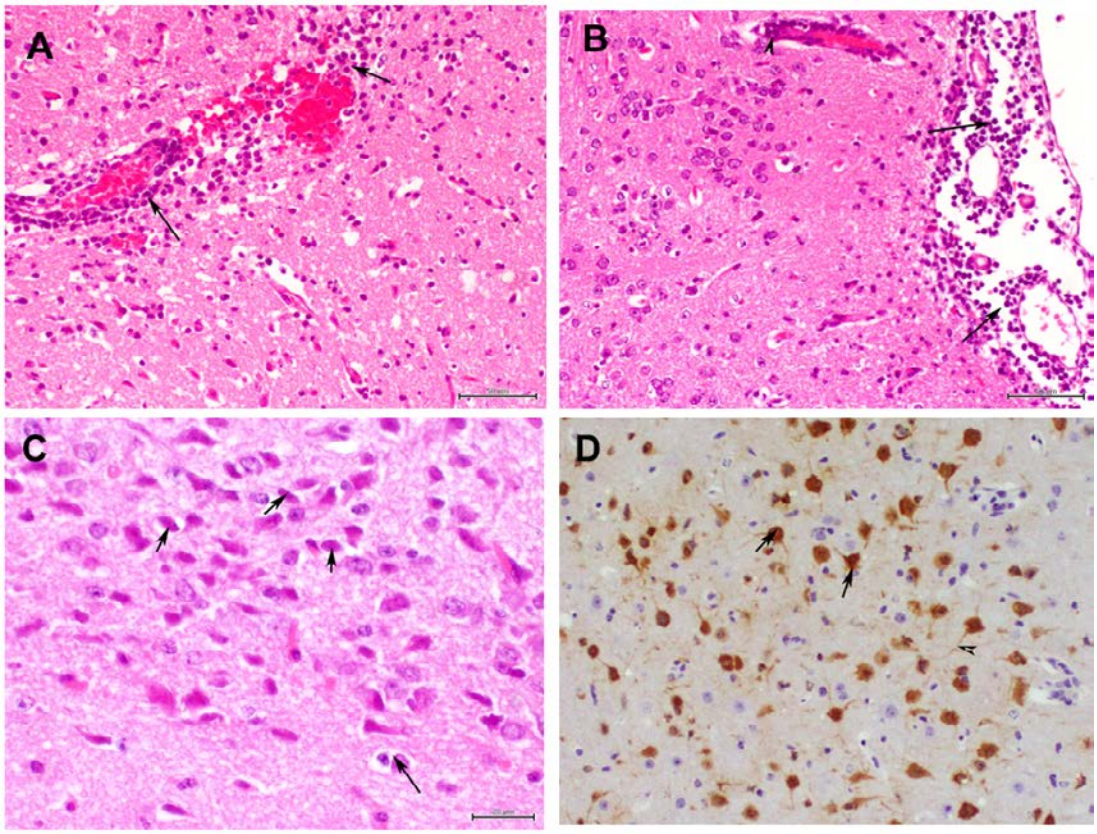

Figure 1: Non-suppurative meningoencephalitis in hamsters infected with EHV-9and propolis treated represented by; (A) perivascular cuffing (arrows);(B) meningoencephalitis (arrows),with also perivascular cuffing (arrowhead), H&E, Bar=50 μm; (C) Neuronal degeneration and necrosis with nuclear shrinkage and vacuolation (arrows). H&E, Bar=20 μm; (D) Immunohistochemistry for viral distribution in the brain. Viral antigens were localized in the neuronal body (arrows), processes arrowhead), Bar=50 μm.

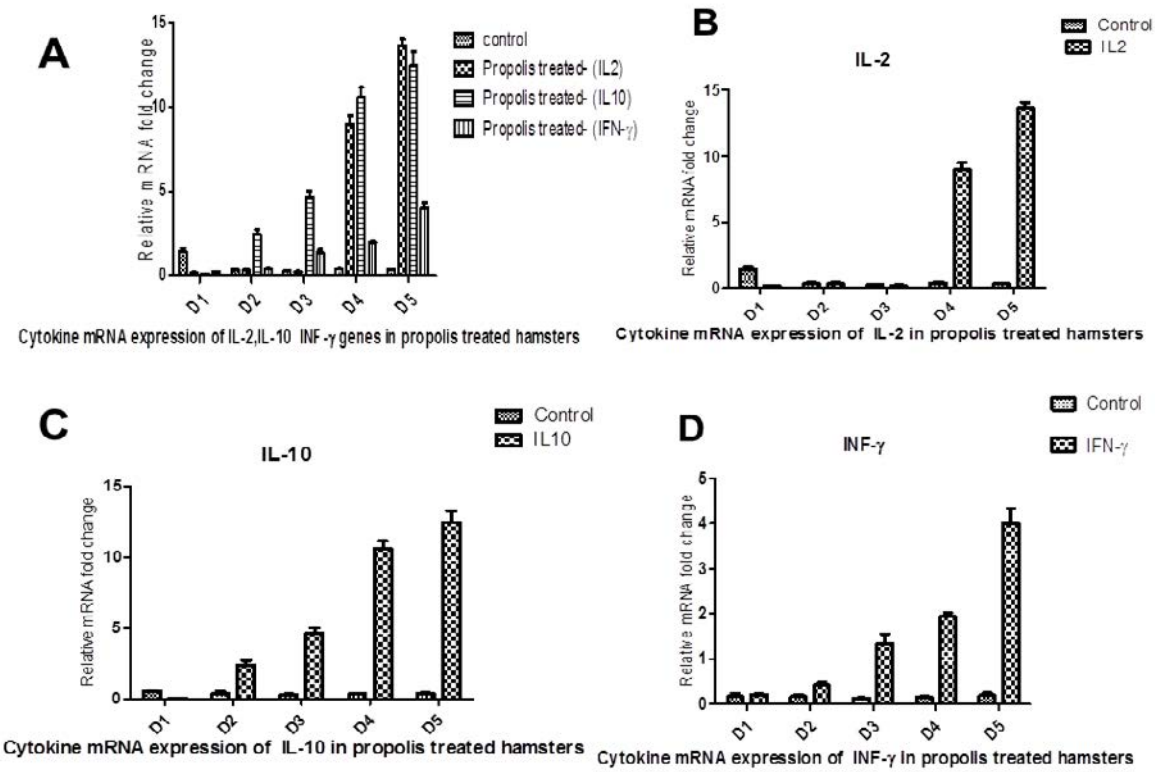

Figure 2: Cytokine mRNA expression in brain tissues of propolis infected hamsters. Target gene expression is presented relative to β-actin expression and normalized to a calibrator. (A) Represent the relative increase of IL2, IL-10.IFN-γ in relation to the control un infected hamsters at D1, D2, D3, D4PI. (B) IL-2, (C) IL-10, (IFN-γ) respectively. The difference between both groups was assessed by two-way ANOVA analysis of variance. Comparisons were considered significant at $P \leq 0.05$.

Discussion

Being a neuropathogenic virus, EHV-9 is replicated in the olfactory mucosal cells after intranasal inoculation [23,24]. The time course of infection from the nasal cavity to the brain, and the characteristics of the brain lesions were similar to that observed in previous studies using a hamster model of EHV-9-induced encephalitis [23,24].

Several studies *in vitro* have shown the effect of propolis on the RNA and DNA of different viruses, including herpes simplex type 1, herpes simplex type 2, adenovirus type 2, poliovirus type 2, vesicular stomatitis virus [7], vaccinia virus, and the virus responsible for Newcastle disease [8]. Flavonoids such as quercetin and rutin, which are found in honey and propolis [25,26], were thought to have antiviral activity against HSV, poliovirus, and Sindbis virus [4,16]. In addition, two flavonoids (chrysin and campherol) have been proven to have a very active role in the inhibition of replication of several herpes viruses, adenovirus, and rotavirus [27]. However, propolis and its derivatives have been reported to possess the ability to inhibit virus propagation via reduction of viral multiplication and virucidal action. In the present study, no obvious effects in preventing EHV-9-induced acute encephalitis were observed after administration of propolis.

A previous study reported that, propolisas a potential immune-enhancing agent via activation of cellular and humoral immunity, as well as a possible natural antibiotic with no side effects [28]. In this study, the concentrations of IL-2, IL-10 and IFN-γ in the brain tissue were determined by qRT-PCR and ΔΔCt method. There were considerable increases in the mRNAs for the Th-1 cytokines, IL-2 and IFN-γ and Th2 cytokines (IL-10) in propolis-treated group before viral challenge in comparison to those of the control group. IL-2, a cytokine secreted by type 1 helper T cells (Th1 cells), mediates cellular immunity. In addition, IL-2 can stimulate the production of immunoglobulins and promote the proliferation and differentiation of natural killer cells [18]. IFN- is responsible for activation of macrophage, killing of many of intracellular pathogens and control of infection [29,30]. IFN-γ is secreted by type 1 helper T cells (Th1 cells), and it can reflect the basic status of cellular and humoral immunity of an organism to a certain extent.

The levels of cytokine secretion reflect the state of cellular and humoral immunity in an organism. Both cellular and humoral immunity play important roles in defense against infectious disease in the host [31]. However, this immune enhancement induced by propolis, encephalitis due to EHV-9 occurred as this virus enters the brain via the olfactory nerve, spreads trans-synaptically to connecting neurons along the olfactory tract, and replicates in the neurons of the olfactory bulbs and cerebrum [23,24].

In the hamster model of herpes virus infection used in the present study, the time course of encephalitis from nasal inoculation of EHV-9 to fulminant encephalitis was rather short. Therefore, there might not have been enough time for progressive enhancement of immune system to protect against EHV-9-induced fulminate encephalitis or at least to decrease the severity of infection after propolis administration [32,33]. Pre-treatment with propolis did not prevent EHV-9-induced acute rhinitis and virus replication in the nasal cavity, and eventually, the virus gained access to the olfactory bulb and cerebrum in

hamsters. The present regime and duration of propolis administered in this study was inadequate for preventing virus replication or viral induced encephalitis or both, however its ability to enhance immunity. Further studies with prolonged course of treatment and larger doses are in progress.

The present study showed that propolis could not necessarily prevent acute encephalitis induced by neuropathogenic EHV-9 by improving cellular and humoral immunity. This may be due to the quick transmission of virus directly through the olfactory nerve to the brain before acquisition of protective immunity. Further study is needed to determine the potential of propolis in preventing EHV-9 in chronic transmission experiments or by using other routes of infection, such as oral or ocular routes.

Acknowledgements

We would like to thank the Nagaragawa Research Center, API (Gifu, Japan) for providing valuable advice and for the supply of propolis.

References

1. Öztürk F, Kurt E, Emiroğlu L, Sobaci G (1999) Effect of propolis on endotoxin-induced uveitis in rabbits. Jpn J Ophthalmol 43: 285-289.
2. Duran N, Muz M, Culha G, Duran, G, Ozer B (2011) GC-MS analysis and antileishmanial activities of two Turkish propolis types. Parasitol Res 108: 95-105.
3. Nina N, Quispe C, Jiménez-Aspee F, Theoduloz C, Feresín GE, et al. (2015) Antibacterial activity, antioxidant effect and chemical composition of propolis from the Regióndel Maule, central Chile. Molecules 20: 18144-18167.
4. Oršolić N, Terzić S, Šver L, Bašić I (2005) Honey-bee products in prevention and/or therapy of murine transplantable tumours. J Sci Food Agric 85: 363-370.
5. Schnitzler P, Neuner A, Nolkemper S, Zundel C, Nowack H, et al. (2010) Antiviral activity and mode of action of propolis extracts and selected compounds. Phytother Res24 :20-28.
6. Silva-Carvalho R, Baltazar F, Almeida-Aguiar C (2015) Propolis: a complex natural product with a plethora of biological activities that can be explored for drug development. Evid Based Complement. Alternat Med 2015: 29.
7. Amoros M, Sauvager F, Girre L, Cormier M (1992) In vitro antiviral activity of propolis. Apidologie 23: 231-240.
8. Maksimova-Todorova V, Gegova G, Serkedzhieva Y, Uzunov S, Pancheva S, et al. (1985) Antiviral effects of some fractions isolated from propolis. Acta Mic Bulg17: 79-85.
9. Bonvehí JS, Bermejo FO (2013) Element content of propolis collected from different areas of South Spain. Environ Monit Assess 185: 6035-6047.
10. Marcucci MC, Ferreres F, Garcıa-Viguera C, Bankova VS, De Castro SL, et al. (2001) Phenolic compounds from Brazilian propolis with pharmacological activities. J Ethnopharmacol 74: 105-112.
11. Gao W, Wu J, Wei J, Pu L, Guo C, et al. (2014) Brazilian green propolis improves immune function in aged mice. J ClinBiochem Nutr 55: 7-10.
12. Sforcin J (2007) Propolis and the immune system: A review. J Ethnopharmacol 113: 1-14.
13. Fukushi H, Tomita T, Taniguchi A, Ochiai Y, Kirisawa R, et al. (1997) Gazelle herpesvirus 1: a new neurotropic herpesvirus immunologically related to equine herpesvirus 1. Virology 227: 34-44.
14. Yanai T, Sakai T, Fukushi H, Hirai K, Narita M, et al (1998) Neuropathological study of gazelle herpesvirus 1 (equine herpesvirus 9) infection in Thomson's gazelles (Gazella thomsoni). J Comp Pathol 119: 159-168.
15. El-Habashi N, El-Nahass ES, Namihira Y, Hagiwara H, Fukushi H (2011) Neuropathogenicity of equine herpesvirus 9 in cattle. J Equine Vet Sci 31: 72-77.
16. Taniguchi A, Fukushi H, Matsumura T, Yanai T, Masegi T, et al. (2000) Pathogenicity of a new neurotropic equine herpesvirus 9(gazelle herpesvirus 1) in horses. J Vet Med Sci 62: 215-218.
17. Taniguchi A, Fukushi H, Yanai T, Masegi T, Yamaguchi T, et al. (2000) Equine herpesvirus 9 induced lethal encephalomyelitis in experimentally infected goats. Arch Virol 145: 2619-2627.
18. Yanai T, Fujishima N, Fukushi H, Hirata A, Sakai H, et al. (2003) Experimental infection of equine herpesvirus 9 in dogs. Vet Pathol 40: 263-267.
19. Saleh AG, Anwar SI, Abas OM, Abd-Ellatieff HA, Nasr M, et al. (2017) Effect of a single point mutation on equine herpes virus 9 (EHV-9) neuropathogenicity after intranasal inoculation in a hamster model. J Vet Med Sci 79: 1426-1436.
20. Escobedo G, Palacios-Arreola MI, Olivos A, López-Griego L, Morales-Montor J (2012) Tamoxifen treatment in hamsters induces protection during taeniosis by Taenia solium. BioMed res inter: 10.
21. Vernel-Pauillac F, Merien F (2006) Proinflammatory and immunomodulatory cytokine mRNA time course profiles in hamsters infected with a virulent variant of Leptospira interrogans. Infecand immun 74: 4172-4179.
22. El-Habashi N, Kato Y, El-Nahass E, Fukushi H, Hirata A, et al. (2013) An ocular infection model using suckling hamsters inoculated with equine herpesvirus 9 (EHV-9): kinetics of the virus and time-course pathogenesis of EHV-9-induced encephalitis via the eyes. Vet Pathol 50: 56-64.
23. Fukushi H, Taniguchi A, Yasuda K, Yanai T, Masegi T, et al. (2000) A hamster model of equine herpesvirus 9 induced encephalitis. J Neurovirol 6: 314-319.
24. Narita M, Uchimura A, Kawanabe M, Fukushi H, Hirai K (2001) Invasion and spread of equine herpesvirus 9 in the olfactory pathway of pigs after intranasal inoculation. J Comp Pathol 124: 265-272.
25. Oršolić N, Bašić I (2005) Water-soluble derivative of propolis and its polyphenolic compounds enhance tumoricidal activity of macrophages. J Ethnopharmacol 102: 37-45.
26. Yao L, Jiang Y, D'Arcy B, Singanusong R, Datta N, et al. (2004) Quantitative high-performance liquid chromatography analyses of flavonoids in Australian Eucalyptus honeys. J Agric Food Chem 52: 210-214.
27. Chen PC, Wong G (1996) Honey bee propolis: prospects in medicine. Bee World 77: 8-15.
28. Fischer G, Conceicao FR, Leite FPL, Dummer LA, Vargas GDA, et al. (2007) Immunimodulation produced by a green propolis extract on humoral and cellular responses of mice immunized with SuHV-1. Vaccine 25: 1250-1256.
29. Heinzel FP, Sadick MD, Holaday BJ, Coffman R, Locksley RM (1989) Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. J Exp Med 169: 59-72.
30. Squires K, Schreiber R, McElrath M, Rubin B, Anderson S, et al. (1989) Experimental visceral leishmaniasis: role of endogenous IFN-gamma in host defense and tissue granulomatous response. The J Immunol 143: 4244-4249.
31. Fischer G, Conceicao FR, Leite FPL, Dummer LA, Vargas GDA, et al. (2007) Immunimodulation produced by a green propolis extract on humoral and cellular responses of mice immunized with SuHV-1. Vaccine 25: 1250-1256.
32. Middleton E (1986) The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer.
33. Nhiem NX, Kiem PV, Minh CV, Tai BH, Tung NH, et al. (2010) Structure-activity relationship of lupane-triterpene glycosides from Acanthopanaxkoreanum on spleen lymphocyte IL-2 and IFN-γ. Bioorg. Med Chem Lett 20: 4927-4931.

Author Affiliation

Top

[1]*Laboratory of Veterinary Pathology, Department of Veterinary Medicine, Gifu University, 1-1 Yanagido, Gifu, Japan 501-1193*

[2]*Department of Pathology and Parasitology, Faculty of Veterinary Medicine, Damanhour University, El-Beheira, Egypt*