Trop J Nat Prod Res, January 2025; 9(1):306 – 310

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Potential Effect of Propolis on Norovirus Viral Load: An Experimental Research using Balb/c Mice

Warda E. Rusdi[1,2], Soetjipto[3,4]*, Maria I. Lusida[4,5], Irmawan Farindra[6], Bambang E. Suwito[6], Muhammad S. Rusdi[7], Wilhemus D.M.R Benge[8], Irsandi R. Farmananda[9], Mahdiyah H. Hanifa[10], Hidayat[10]

[1]*Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya Indonesia.*
[2]*Department of Public Health, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia.*
[3]*Department of Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[4]*Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.*
[5]*Department of Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[6]*Department of Anatomy and Histology, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia.*
[7]*Department of Medical Research, Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jawa Timur.*
[8]*Universitas Pembangunan Nasional "Veteran" Jawa Timur.*
[9]*Department of Anatomy, Faculty of Medicine, Universitas Islam Al-Azhar Mataram, Mataram, Indonesia.*
[10]*Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia.*

ARTICLE INFO

Article history:
Received 29 October 2024
Revised 31 October 2024
Accepted 30 November 2024
Published online 01 February 2025

ABSTRACT

Norovirus infection is a significant public health concern, particularly among children, due to its association with diarrhea. The current management strategies for Norovirus primarily focus on rehydration and symptomatic relief. This research was performed to examine the effects of propolis on the viral load and the severity of diarrhea caused by Human Norovirus (HuNoV) infection. In this experimental research, a total of 21 BALB/c mice were randomly assigned to three groups: Group K(-) (control group), Group K(+) (exposed solely to HuNoV), and Group P (exposed to HuNoV with propolis intervention). A 1 ml suspension of HuNoV was administered to the mice via the intraperitoneal route. Rapid Diagnostic Tests (RDT) Norovirus Kit and Real-Time Polymerase Chain Reaction (RT-PCR) were performed 48 hours post-infection to confirm successful viral infection. The severity of diarrhea was assessed using the paper towel method. Propolis was administered as a suspension at a dosage of 2.5 mg/kg body weight for five days. On day eight, the mice were euthanized, and fecal samples were collected from the colon for further analysis. RT-PCR was conducted again to quantify the viral load. The results on day eight indicated a significant reduction in viral load in Group P, whereas Group K(-) showed no such reduction. Furthermore, the severity of diarrhea in the mice treated with propolis was found lower. These findings suggest that propolis contributes to the reduction of viral load and the diarrhea severity in mice infected with Norovirus.

Keywords: Human Norovirus, Diarrhea, Degree Severity, Propolis, Mice

Introduction

In recent decades, scientific research has extensively identified viruses and bacteria as the primary causes of various complications and disorders within the human digestive system. Norovirus has been recognized as a major pathogen that significantly contributes to gastrointestinal issues, particularly among children.[1,2] Norovirus often causes disease outbreaks in high-density settings, including schools, daycare centers, and healthcare facilities.[3,4] In addition to younger population, the elderly population is also highly vulnerable with high mortality and morbidity associated with Norovirus infection.[5,6] Comprehensive global research estimates the prevalence of Norovirus infection of approximately 19.04%,[2] which percentage varies across regions.

*Corresponding author. E mail: soetjipto@fk.unair.ac.id
Tel: + (+62) 877-8491-1731

Citation: Rusdi WE, Soetjipto, Lusida MI, Farindra I, Suwito BE, Rusdi MS, Benge WDMR, Farmananda IR, Hanifa MH, Hidayat.The Potential Effect of Propolis on Norovirus Viral Load: An Experimental Research using Balb/c Mice. Trop J Nat Prod Res. 2024; 9(1): 306 - 310
https://doi.org/10.26538/tjnpr/v9i1.39

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

A study by Qi and Colleagues in 2018 reported that Africa shows the highest prevalence rate of 15%, while Europe and North America exhibit the lowest rates at 4%.[7] Despite significant advances in research, data regarding the incidence and prevalence of Norovirus infection remain limited.[8] Norovirus infection can be prevented and treated using antimicrobiotics, antioxidant, and anti-inflammatory substances. Propolis is a resinous substance bees produce from plant sap that contains antimicrobial, antioxidant, and anti-inflammatory properties.[9,10] Propolis has a wide range of antiviral activities effective against numerous viral pathogens, including herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), influenza viruses A and B, human immunodeficiency virus (HIV), and SARS-CoV-2, the virus responsible for the COVID-19 pandemic, among others.[11–14] Propolis also has antibacterial activity. A study by Megantara and colleagues in 2024 showed that routine administration of propolis for 12 weeks was able to inhibit the growth of *E. coli* bacteria. Through this study, it also acts as an immunomodulator that stimulates IL-6 levels to overcome inflammation due to bacterial infections.[15] Flavonoids, including quercetin, kaempferol, and apigenin, are recognized as key chemical constituents that significantly contribute to the antiviral properties of propolis.[16,17] In addition to these flavonoids, phenolic acids, such as caffeic acid and chlorogenic acid, enhance the antiviral efficacy of propolis, thereby contributing to its overall therapeutic potential against a wide range of viral infections that impact human health.[18–20] This

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

research aims to investigate and assess the effects of propolis administration on Norovirus viral load in mice with diarrhea. Furthermore, it seeks to explore the potential advantages or disadvantages of using propolis in such medical contexts.

Material and Methods

Experimental Animal and Ethical Clearance

A total of 21 male BALB/c mice, aged 8 weeks and weighing 25 grams, were obtained from the Pharmacology Laboratory at the Faculty of Medicine, Airlangga University, Indonesia. The mice were acclimatized in the laboratory for 7 days to stabilize their homeostasis prior to the experiment. Each mouse was housed individually in a cage measuring 15 cm x 25 cm x 15 cm, with the cages arranged on a shelf by group. The animals were provided with water and standard feed ad libitum under a 12-hour light/dark cycle. All procedures involving the experimental animals were reviewed and approved by the Health Research Ethics Committee of the Faculty of Medicine, Airlangga University (Approval No: 65/EC/KEPK/FKUA/2024).

Experimental design

This true experimental research was performed in three groups of mice (7 mice per group) that were randomly assigned to different treatments. Group K(-) served as the control group, receiving neither a HuNoV injection nor any intervention. Group K(+) was injected with HuNoV but did not receive any further treatment. Lastly, group P was both injected with HuNoV and administered propolis at a dose of 2.5 mg/kg body weight for 5 days.

HuNoV sample

A 25 ml suspension of HuNoV was obtained from the Institute of Tropical Disease Center, Airlangga University. Before use, the virus titer was quantified using qPCR, and set at 10^5. The viral strain was further characterized by sequencing the RdRp (ORF1) and the junction regions of ORF ½. The HuNoV suspension was prepared in a phosphate-buffered saline (PBS) solution at a concentration of 10% w/v, and then clarified through centrifugation. The virus suspension was stored in a 10 ml Eppendorf tube, kept at -80°C, and subsequently transported to the laboratory for the experiment.

HuNoV Administration

The administration of the HuNoV suspension to the mice followed the procedure outlined by Rusdi and colleagues in previous research in 2024.[21] Before the initiation of the infection protocol, fecal specimens from all acclimatized mice were examined utilizing the Rapid Diagnostic Test (RDT) Norovirus Kit (Bionevan Co. Ltd) to ascertain their absence of Norovirus infection. The evaluation was performed by procuring fecal specimens from each individual mouse within the entire group. The frozen HuNoV suspension was thawed by centrifuging at 5,300 g for 5 minutes at 15°C. The virus was injected intraperitoneally in the posterior quadrant of the abdomen at a dose of 1 cc of the HuNoV suspension for all groups. Forty-eight hours after infection, a re-examination was conducted to verify successful HuNoV infection in the K (+) and P group.

Propolis Extract

A commercially available propolis product manufactured by PT. Melia Sehat Sejahtera in a 6 ml preparation was used, with each 1 ml containing 150 mg of propolis. The preparation was subsequently reconstituted into a suspension, with the dosage adjusted for the experimental animals. The dose calculation followed the method outlined by Nair and Jacob.[22] Propolis was administered to the mice at a dose of 2.5 mg/kg body weight orally via gavage for 7 days post-infection.

Diarrhea Degree Severity

The severity of diarrhea in the mice was assessed using the method described by Kim and colleagues, where observations can be effectively made by placing tissue paper at the bottom of the cage, which provides a clear image of the mouse feces with minimal disturbance. The severity of diarrhea was categorized based on fecal condition into four levels: (+) wet feces, (++) pale feces, (+++) semi-liquid feces, and (++++) runny feces.[23]

Stool Extraction

Fecal examination of the mice was conducted on day 8. The procedure began with anesthesia administered via the intraperitoneal route using ketamine at a dose of 0.05 mL. A surgical procedure followed, involving a transverse incision in the lower central abdominal region, extending superiorly. The spleen was carefully separated from surrounding tissues, and the ileum was excised, from the ileocecal junction to approximately 2-3 centimeters proximally. Fecal specimens were collected from sections of the descending and transverse colon. The samples were placed in sterile containers, stored at -80°C, and then sent to the Viral Diarrhea Laboratory at the Institute of Tropical Disease Center, Universitas Airlangga for further analysis.

RT-PCR Examination

A fecal sample was meticulously collected and subjected to a detailed analysis aimed at detecting Norovirus using RT-PCR, specifically targeting the GII strain to accurately measure the viral titer present in the sample. The RT-PCR procedure was conducted in strict accordance with established protocols, ensuring that all steps were meticulously followed to maintain the integrity and reliability of the results. A 10% fecal suspension was prepared by diluting the sample in distilled water, followed by centrifugation at 21,130 g for 10 minutes. Subsequently, the extraction of RNA was meticulously carried out from a total volume of 140 microliters of the supernatant that had been previously obtained, utilizing the highly efficient QIAamp microspin columns specifically designed for such molecular biology applications. This process facilitated the effective separation of components by density, enabling thorough analysis of both the sediment and supernatant for viral detection. The probe primers used for GII screening of HuNoV strains are shown in Table 1.

Table 1: RT-PCR primers probe used for HuNoV strain GII screening

Genogroup	Primer of Probe	Sequence (5'→3')[a]	Polarity[b]	Location
GII	Primer COG2F	CARGARBCNATGTTYAGRTGGATGAG	+	5003
	Primer COG2R	TCGACGCCATCTTCATTCACA	-	5100
	Primer RING-TP	FAM-TGGGAGGGCGATCGCAATCT-TAMRA	+	5048

Results and Discussion

A total of 21 BALB/c mice were utilized in this experimental research. On the first day following the acclimatization period, 1 ml of HuNoV suspension in phosphate-buffered saline (PBS) was administered to all mice in the K(+) and P1 groups via the intraperitoneal route. The initial examination was conducted 48 hours post-infection using the RDT Norovirus Kit, which yielded positive results. To further confirm the

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

successful HuNoV infection in the mice, additional testing was performed using RT-PCR on fecal samples collected from the mice. The examination results indicated an increase in viral load, reaching 10^5 in all fecal samples from the K(+) and P1 group, confirming that all mice were infected with HuNoV (Table 2). The severity of diarrhea in the mice was monitored starting from the first-day post-infection. Measurement of diarrhea severity was conducted by observing feces adhering to tissue paper placed at the bottom of the cage. In the K(+) group, one day after infection, most mice exhibited diarrhea classified as (+), while only one mouse showed symptoms in the (++++) category. On the third day, one mouse succumbed, likely due to an inability to withstand the high titers of Norovirus, resulting in severe diarrhea. From that day onward, the severity of diarrhea in the remaining mice continued to escalate.

Table 2. Summary of RT-PCR and RDT results

	Sample ID	48h Post Infection			Day 8		
		Rapid Test	RT-PCR		Rapid Test	RT-PCR	
			Call	Viral Load		Call	Viral Load
HNV (-)	K-1	*Neg	Neg	ND	Neg	Neg	**ND
	K-2	Neg	Neg	ND	Neg	Neg	ND
	K-3	Neg	Neg	ND	Neg	Neg	ND
	K-4	Neg	Neg	ND	Neg	Neg	ND
	K-5	Neg	Neg	ND	Neg	Neg	ND
	K-6	Neg	Neg	ND	Neg	Neg	ND
	K-7	Neg	Neg	ND	Neg	Neg	ND
HuNoV (+) Propolis (-)	K+1	***Pos	Pos	10^5	Pos	Pos	10^5
	K+2	Pos	Pos	10^5	Pos	Pos	10^5
	K+3	Pos	Pos	10^5	Pos	Pos	10^5
	K+4	Pos	Pos	10^5	Pos	Pos	10^5
	K+5	Pos	Pos	10^5	Pos	Pos	10^5
	K+6	Pos	Pos	10^5	Pos	Pos	10^5
	K+7	Pos	Pos	10^5	Pos	Pos	10^5
HuNoV (+) Propolis (+)	P1.1	Pos	Pos	10^5	Neg	Neg	ND
	P1.2	Pos	Pos	10^5	Neg	Neg	ND
	P1.3	Pos	Pos	10^5	Neg	Neg	ND
	P1.4	Pos	Pos	10^5	Neg	Neg	ND
	P1.5	Pos	Pos	10^5	Neg	Neg	ND
	P1.6	Pos	Pos	10^5	Neg	Neg	ND
	P1.7	Pos	Pos	10^5	Neg	Neg	ND

*ND = No viral load was detected
**Neg = Negative
***Pos = Positive

On the other side, the P1 group showed changes in fecal characteristics within hours of infection, with some mice displaying semi-liquid diarrhea (+++) and others presenting pale feces (++). The severity of diarrhea increased until the second day after infection. Following the administration of propolis on the third day, noticeable changes in fecal characteristics began to emerge on the fourth day, with most mice producing feces categorized as (++). By the day prior to sacrifice, all mice in this group exhibited feces with characteristics classified as (+). All observation data on the severity of diarrhea in the K(+) and P groups are shown in Figure 1.

The rapid transmission rate of Norovirus with unavailable a vaccine creates significant public health challenges. This research investigates the impact of propolis administration on Norovirus viral load in mice with diarrhea, aiming to comprehensively evaluate the potential of propolis as a treatment option for diarrhea resulting from Norovirus infection. Observations in the P group indicated a decrease in viral load following the administration of propolis at a consistent dose over five consecutive days. This finding suggests that propolis can effectively inhibit viral replication, leading to a reduction in viral load by the fifth day. Such results highlight the antiviral properties of propolis in mitigating viral replication within the host organism.

Propolis exerts an influence on viral replication by obstructing the entry of viruses into host cells and by facilitating the degradation of RNA prior to the entry of the virus.[24] Propolis extracts have demonstrated significant antiviral effects, notably against the SARS-CoV-2 virus, which causes COVID-19, as well as considerable efficacy against various respiratory viruses, including influenza, parainfluenza, and rhinoviruses commonly associated with respiratory illnesses.[12,13] Furthermore, a reduction in viral replication in SARS-CoV-2 infections when treated with 80% extract from poplar propolis was identified by Sberna and colleagues.[25] In vitro research also found virucidal activity of Brazilian green propolis extract against the ACV-resistant AR-29 HSV-1 strain (PE-8) infection.[26]

HuNoV infection has the potential to induce significant alterations in the histological architecture of the human digestive system, primarily due to the inflammation and cellular damage occurring at the microscopic level within the affected tissues. To date, over 500 compounds in propolis have been successfully identified, each possessing a diverse array of properties and health benefits.[27] Phenolic

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

compounds, including flavonoids, exhibit antiviral properties that contribute to the inhibition of viral replication within the host.

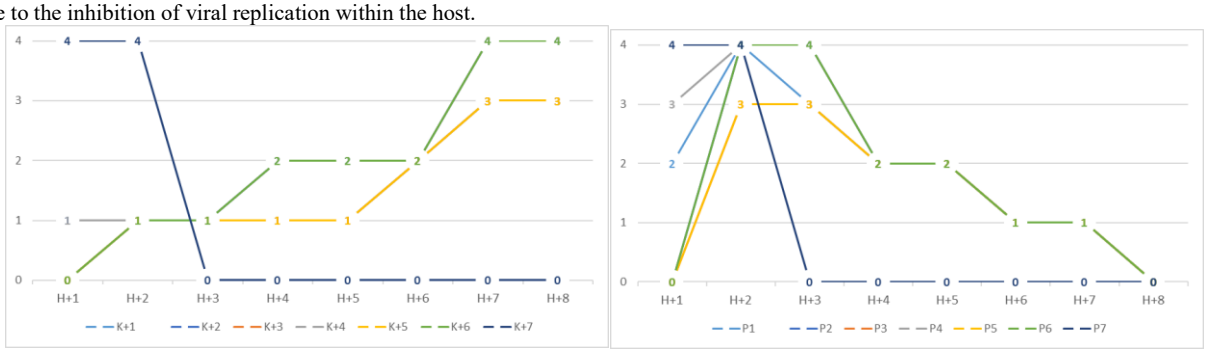

Figure 1: Diarrhea severity in mice. (left) group K(+), (right) group P (propolis intervention). Both in K(+) and P groups, one mice was found death.

Note. Scale 1 indicates category (+), 2 (++), 3 (+++), and 4 (++++). H+1 - H+8 indicates the days after mice were infected with HuNoV.

This inhibition may occur through various mechanisms, including biochemical interactions and cellular pathways that collectively work to suppress viral proliferation and enhance the host's immune response to invading pathogens. The components of propolis serve as a barrier that obstructs the enzymes and proteins required by foreign pathogens to invade host cells.[28] The phenolic acid content in propolis has also been well-documented in previous studies for its antiviral properties against multiple pathogen infections.

Despite the findings of this research, several limitations must be acknowledged, as they may impact the results. Future research should address these limitations comprehensively. Firstly, it is important to note that this research utilized commercially available propolis preparations rather than samples sourced directly from their natural habitat. This choice could introduce variability in the composition of propolis, as specific constituents may undergo alterations during processing and packaging. Secondly, histological examination of the digestive tissues of the mice was not conducted in this research. Future research focusing on histological analysis is essential to better understand the pathophysiology of HuNoV infection in the digestive system.

The potential of propolis as a natural remedy remains broad and warrants further investigation. Beyond its antiviral properties, propolis is also believed to aid in mitigating the effects of pathogen infections. Future studies are expected to elucidate the repair mechanisms that propolis may possess in cases of infection leading to tissue inflammation. Consequently, propolis could serve as an alternative treatment not only for diarrhea caused by Norovirus but also for various other health conditions associated with pathogen infections. Additionally, it should be recognized that the diverse types of propolis found worldwide exhibit unique compositions, influenced by factors such as the botanical resin source, the honey bee species involved, and the extraction methods employed.[29]

Conclusion

This research did not yet examine the mechanisms underlying the propolis effect, yet the use of propolis over a five-day period reduced the viral load associated with Norovirus infection, as evidenced by RT-PCR analysis. A notable decrease in the severity of diarrhea in the mice was also confirmed. Propolis holds a lot of potential against Norovirus infection cases. The findings of this study are the first step to develop a more effective propolis formulation to overcome diarrhea caused by Norovirus infection. Assessing the toxicity and safety aspects of propolis in long-term use is also a challenge in the future, especially in vulnerable populations, such as children and the elderly.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

We would like to thank the Universitas Nahdlatul Ulama Surabaya for their assistance, which enable this research to be done.

References

1. Shah MP, Hall AJ. Norovirus Illnesses in Children and Adolescents. Infect Dis Clin North Am. 2018;32(1):103.
2. Zhang P, Hao C, Di X, Chuizhao X, Jinsong L, Guisen Z, Hui L, Zhaojun D. Global prevalence of norovirus gastroenteritis after emergence of the GII.4 Sydney 2012 variant: a systematic review and meta-analysis. Front Public Health. 2024;12:1373322.
3. Lian Y, Wu S, Luo L, Lv B, Liao Q, Li Z, Rainey J, Hall A, Ran L. Epidemiology of Norovirus Outbreaks Reported to the Public Health Emergency Event Surveillance System, China, 2014–2017. Viruses. 2019;11(4):2014–2017.
4. Carlson KB, Dilley A, O'Grady T, Johnson JA, Lopman B, Viscidi E. A narrative review of norovirus epidemiology, biology, and challenges to vaccine development. Vaccines (Basel). 2024 ;9(1):1–9.
5. Chen Y, Hall AJ, Kirk MD. Norovirus Disease in Older Adults Living in Long-Term Care Facilities: Strategies for Management. Curr Geriatr Rep. 2017;6(1):26–33.
6. Tsai H, Yune P, Rao M. Norovirus disease among older adults. Ther Adv Infect Dis. 2022;9:1–13.
7. Qi R, Huang Y ting, Liu J wei, Sun Y, Sun X feng, Han HJ, Qin XR, Zhao M, Wang LJ, Wenqian L, Li JH, Chen C, Yu XJ. Global Prevalence of Asymptomatic Norovirus Infection: A Meta-analysis. EClinicalMedicine. 2018;2–3:50–58.
8. Thébault A, David J, Kooh P, Cadavez V, Gonzales-Barron U, Pavio N. Risk factors for sporadic norovirus infection: A systematic review and meta-analysis. Microb Risk Anal. 2021;17(100135):1–10.
9. Touzani S, Embaslat W, Imtara H, Kmail A, Kadan S, Zaid H, Elarabi I, Badiaa L, Saad B, Kabir Y. In Vitro Evaluation of the Potential Use of Propolis as a Multitarget Therapeutic Product: Physicochemical Properties, Chemical Composition, and Immunomodulatory, Antibacterial, and Anticancer Properties. Biomed Res Int. 2019;2019(4836378):1–11.

10. Rufatto LC, dos Santos DA, Marinho F, Henriques JAP, Roesch Ely M, Moura S. Red propolis: Chemical
11. Fiorini AC, Scorza CA, de Almeida ACG, Fonseca MCM, Finsterer J, Fonseca FLA, Scorza F. Antiviral activity of Brazilian Green Propolis extract against SARS-CoV-2 (Severe Acute Respiratory Syndrome - Coronavirus 2) infection: case report and review. Clinics. 2021;76(e2357):1–4.
12. Ożarowski M, Karpiński TM. The Effects of Propolis on Viral Respiratory Diseases. Molecules. 2023;28(359):1–14.
13. Magnavacca A, Sangiovanni E, Racagni G, Dell'Agli M. The antiviral and immunomodulatory activities of propolis: An update and future perspectives for respiratory diseases. Med Res Rev. 2022;42(2):897–945.
14. Ghosh S, Al-Sharify ZT, Maleka MF, Onyeaka H, Maleke M, Maolloum A, Godoy L, Meskini M, Rami M, Ahmadi S, Al-Najjar S, Al-Sharify N, Ahmed S, Dehghani M. Propolis efficacy on SARS-COV viruses: a review on antimicrobial activities and molecular simulations. Environ Sci Pollut Res. 2022;29(39):58628–58647.
15. Megantara I, Murad C, Pahlevi F, Sylviana N, Goenawan H, Lesmana R. The Effect of Propolis Extract from Sumatra, Indonesia on *Escherichia coli* and IL-6 Gene Expression in Male Wistar Rats Fed with a High-Fat Diet. Trop J Nat Prod Res. 2024;8(6):7350–7354.
16. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas A, Jaremko M. Important Flavonoids and Their Role as a Therapeutic Agent. Molecules. 2020;25(22):5243.
17. Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, Alshahrani M, Islam S, Islam M. Flavonoids a Bioactive Compound from Medicinal Plants and Its Therapeutic Applications. Biomed Res Int. 2022;2022:1–19.
18. Hassan STS, Šudomová M, Mazurakova A, Kubatka P. Insights into Antiviral Properties and Molecular Mechanisms of Non-Flavonoid Polyphenols against Human Herpesviruses. Int J Mol Sci. 2022;23(22):1–21.
19. Kiokias S, Oreopoulou V. A Review of the Health Protective Effects of Phenolic Acids against a Range of Severe Pathologic Conditions (Including Coronavirus-Based Infections). Molecules. 2021;26(17):1–18.
20. Kurek-Górecka A, Kłósek M, Pietsz G, Czuba ZP, Kolayli S, Can Z, Balwierz R, Olczyk P. The Phenolic Profile and Anti-Inflammatory Effect of Ethanolic Extract of Polish Propolis on Activated Human Gingival Fibroblasts-1 Cell Line. Molecules. 2023;28(22):1–23.
composition and pharmacological activity. Asian Pac J Trop Biomed. 2017;7(7):591–598.
21. Rusdi WE, Soetjipto, Lusida MI, Dinana Z, Maharani AT, Farindra I, Huda N, Maharani R, Hamida A. Mice as Small Animal Model for Human Norovirus Infection. Trop J Nat Prod Res. 2024;8(5):7089–7092.
22. Nair A, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27–31.
23. Yim SK, Kim SW, Lee ST. Efficient Stool Collection Methods for Evaluating the Diarrhea Score in Mouse Diarrhea Models. In Vivo (Brooklyn). 2021;35(4):2115–2125.
24. Yosri N, El-Wahed AAA, Ghonaim R, Khattab OM, Sabry A, Ibrahim MAA, Moustafa M, Gou Z, Zou X, Algethami A, Masry S, Alajmi M, Afifi H, Khalifa S, El-Seedi H. Anti-Viral and Immunomodulatory Properties of Propolis: Chemical Diversity, Pharmacological Properties, Preclinical and Clinical Applications, and In Silico Potential against SARS-CoV-2. Foods. 2021;10(8):1–47.
25. Sberna G, Biagi M, Marafini G, Nardacci R, Biava M, Colavita F, Piselli P, Miraldi E, D'Offizi G, Capobianchi M, Amendola A. In vitro Evaluation of Antiviral Efficacy of a Standardized Hydroalcoholic Extract of Poplar Type Propolis Against SARS-CoV-2. Front Microbiol. 2022;13(799546):1–15.
26. Mazia RS, De Araújo Pereira RR, De Francisco LMB, Natali MRM, Dias Filho BP, Nakamura C, Bruschi M, Ueda-Nakamura T. Formulation and Evaluation of a Mucoadhesive Thermoresponsive System Containing Brazilian Green Propolis for the Treatment of Lesions Caused by Herpes Simplex Type I. J Pharm Sci. 2016;105(1):113–121.
27. Peixoto M, Freitas AS, Cunha A, Oliveira R, Almeida-Aguiar C. Antioxidant and antimicrobial activity of blends of propolis samples collected in different years. LWT. 2021;145:111311.
28. Zulhendri F, Lesmana R, Tandean S, Christoper A, Chandrasekaran K, Irsyam I, Suwantika A, Abdulah R, Wathoni N. Recent Update on the Anti-Inflammatory Activities of Propolis. Molecules. 2022;27(8473):1–61.
29. Chuttong B, Lim K, Praphawilai P, Danmek K, Maitip J, Vit P, Wu M, Ghosh S, Jung C, Burgett M, Hongsibsong S. Exploring the Functional Properties of Propolis, Geopropolis, and Cerumen, with a Special Emphasis on Their Antimicrobial Effects. Foods. 2023;12(21):3909.