Strong anti-SARS-CoV-2 activity of *Lucilia cuprina* maggots’ excretion/secretion and its effect on viral entry and notch pathway *in vitro*: First work

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**ABSTRACT**

The global pandemic caused by SARS-CoV-2 requires new lines of treatment to hinder viral entry and pathogenesis. *Lucilia cuprina* maggots’ excretion/secretion (E/S) contains proteases and antioxidants, among other active ingredients that contribute to its antibacterial, antifungal, and antiviral activity. This study aims to assess the potential effects of E/S on the entry and molecular pathogenesis of a SARS-CoV-2 isolate “NRC-03-nhCoV” *in vitro* for the first time. E/S was obtained from the collected maggots of *L. cuprina* that were maintained under controlled laboratory conditions. The E/S was used to treat VERO-E6 cells infected with SARS-CoV-2. The predicted antiviral activity of the E/S and the expression of the Notch pathway and viral pathogenesis-related genes were assessed at three time points. E/S showed potential antiviral activity against SARS-CoV-2 (IC\textsubscript{50} = 0.324 µg/ml) with a high selectivity index value (SI = 572.997). Serine protease present in E/S was predicted to interact with transmembrane protease, serine 2 and cathepsin B. E/S was able to significantly downregulate Notch-related genes, SUMO1, and TDG in SARS-CoV-2-infected cells, shifting their expression toward levels of the control.

Therefore, E/S of *L. cuprina* maggots is a potential strong inhibitor for SARS-CoV-2.

**INTRODUCTION**

SARS-CoV-2 is causing a global pandemic of the respiratory disease coronavirus disease 2019 (COVID-19), with multiple acute and long-term complications (Chen *et al.*, 2020). Since the COVID-19 pandemic outbreak from Wuhan, China, scientists have been looking for effective vaccines or novel antiviral agents for the SARS-CoV-2 virus (Kandeil *et al.*, 2021; Mahmoud *et al.*, 2020). Scientists were attracted to a variety of natural products of different sources and organisms that can provide new opportunities for the treatment and prevention of infectious diseases (Khare *et al.*, 2020). More than 70% of living organisms are represented by insects (Loram, 2006). Insects have long been used as food, medication, and chemical products due to their abundance and variety in the human environment. For more than 2,000 years, medicinal insects and their products have been used as part of Chinese medicine to treat ailments, whether directly or indirectly, and insect-related drugs may be derived from adult insects or larvae (Feng *et al.*, 2009). Maggots (fly larvae), especially *Lucilia sericata* and *Lucilia cuprina* maggots, were used in wound treatments (Hassan *et al.*, 2014; Sherman, 2009). The maggots produce excretion/secretion (E/S) containing serine...
proteases and antioxidants (such as cysteine and glutathione), among other active ingredients (Casu et al., 1994; Chambers et al., 2003; Gupta, 2008; Nigam et al., 2006; Vistnes et al., 1981). E/S has effects against bacteria (Jiang et al., 2012; Van der Plas et al., 2008), fungi (Pöppel et al., 2014), and viruses (Abdel-Samad, 2019).

The viral entry of SARS-CoV-2 was shown to be through binding of its spike protein (S-protein) with angiotensin-converting enzyme 2 (ACE2) receptors on the cell membrane facilitated by the transmembrane protease, serine 2 (TMPRSS2). TMPRSS2 was shown to proteolytically cleave and activate the SARS-CoV-2 S-protein, among many other viruses, promoting viral uptake (Ragia and Manolopoulos, 2020). Another pathway of viral entry utilizes cathepsin B (endosomal pathway), which is a cysteine protease expressed in endosomes and acts by facilitating the fusion of the viral and endosomal membrane. Cathepsin B works independently from TMPRSS2 (Padmanabhan et al., 2020). Targeting TMPRSS2 and/or cathepsin could hinder viral entry and decrease viral load.

The viral infection was shown to dysregulate multiple pathways related to immunity, protein degradation, blood coagulation, neuronal functions, among many others. One of the important pathways involved in immunity is the Notch signaling pathway which affects the differentiation of the lymphoid T and B cell lineages. The Notch path was proved to be involved in several bacterial and viral infections via multiple mechanisms affecting inflammation and several cell signaling pathways (Ito et al., 2011; Keewan and Naser, 2020; Lu et al., 2020), including the pathogenesis of SARS-CoV-2 (Breikaa and Lilly, 2021).

Protein degradation pathways such as ubiquitination, SUMOylation, and Neddylation were reported to be dysregulated in viral infections (Masucci, 2020). In SARS-CoV-2 infection, the SUMOylation and Neddylation pathways were previously shown to be dysregulated (Ibrahim and Ellakwa, 2021). SUMO could be a potential target in the treatment of SARS-CoV-2 infection (Ryu, 2021).

One of the important defense defense mechanisms of host cells against viral infection is enzymatic deamination of viral DNA by host cells. The base excision repair gene TDG glycosylases was reported to be dysregulated in viral infection (Pytel et al., 2008). TDG is known to affect immunity, particularly innate immunity (Jacobs and Schär, 2012). It is also known to be involved in SUMO signalling signaling (Smet-Nocco et al., 2011).

Inhibition of the TMPRSS2 and cathepsin pathways was shown to be effective against coronaviruses infection (Kawase et al., 2012) and in SARS-CoV-2 infection (Ragia and Manolopoulos, 2020). This study aims to assess the potential efficacy of L. cuprina maggots’ E/S in preventing cell entry of SARS-CoV-2 infection and progression of COVID-19 for the first time. It also aims to assess its effect on key genes and pathways related to SARS-CoV-2 pathogenesis.

MATERIALS AND METHODS

E/S collection

Lucilia cuprina maggots’ E/S was obtained using procedures described in the literature (Abdel-Samad, 2019). In brief, L. cuprina maggots in their third larval instar were isolated from a maintained laboratory culture of L. cuprina at the Animal House, Faculty of Science, Al-Azhar University. The isolated maggots were washed with 70% ethyl alcohol followed by distilled water, before being incubated in darkness with phosphate-buffered saline for 6 hours at 25°C. The E/S was collected, centrifuged, filtrated, and stored at −20°C.

**In vitro antiviral activity**

**Cells and virus**

In a humidified incubator at 37°C and 5% CO₂, the VERO-E6 cells (ATCC® CRL-1586™) were suspended in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 1% penicillin/ streptomycin and 10% fetal bovine serum. The SARS-CoV-2 “NRC-03-nhCoV” virus (Kandeil et al., 2020) was propagated and titrated as previously described (Mostafa et al., 2020).

**Cytotoxicity determination**

Half-maximal cytotoxic concentration (CC₅₀) of L. cuprina was assessed in VERO-E6 cells using the crystal violet assay as previously described (Feoktistova et al., 2016). Briefly, L. cuprina maggots’ E/S stock solutions were prepared in 10% Dimethyl sulfoxide (DMSO) in ddH₂O; several dilutions of this solution were prepared using DMEM. In parallel, the cells were seeded in 96-well plates (100 µl/well, density of 3 × 10⁵ cells/ml) and incubated for 24 hours in a 5% CO₂ humidified incubator at 37°C. After the incubation period, cells were treated with various concentrations of the E/S in triplicate. After 72 hours, the supernatant was discarded, and cell monolayers were fixed using 10% formaldehyde for 1 hour at room temperature (RT). The fixed monolayers were then dried and stained with 50 µl of 0.1% crystal violet for 20 minutes on a bench rocker at RT. The stained cell monolayers were then washed with 70% ethyl alcohol followed by distilled water, before being incubated in darkness with phosphate-buffered saline for 6 hours at 25°C. The E/S was collected, centrifuged, filtrated, and stored at −20°C.

Inhibitory concentration determination

The half-maximal inhibitory concentration (IC₅₀) value for L. cuprina E/S was determined as previously described (Mostafa et al., 2020), with minor modifications. Briefly, VERO-E6 cells were seeded in 96-well tissue culture plates (2.4 × 10⁴ well) and incubated overnight in a 5% CO₂ humidified incubator at 37°C. The cell monolayers were then washed using 1 × phosphate buffer saline (PBS). An aliquot of the SARS-CoV-2 virus containing 100 TCID₅₀ was incubated with serial dilutions of L. cuprina E/S and kept at 37°C for 1 hour. The VERO-E6 cells were treated with the virus alone or virus/L. cuprina E/S mixture and incubated at 37°C in a total volume of 200 µl per well. Cell control was cells that were not treated and not infected, and untreated cells infected with virus represented virus control. After incubation in a 5% CO₂ incubator for 72 hours at 37°C, cells were fixed using 10% paraformaldehyde for 20 minutes and stained using 0.5% crystal violet in ddH₂O for 15 minutes at RT. Crystal violet was then dissolved using absolute methanol (100 µl/well), and the absorbance of crystal violet solutions was measured at λ₅₇₀ nm using a multiwell plate reader. The IC₅₀ of the compound is
the concentration required to reduce the virus-induced cytopathic effect by 50%, relative to the virus control.

**Plaque infectivity assay**

To determine the impact of E/S on monocyte (12 hours after infection) and multicyle replication (24–36 hours after infection) kinetics of SARS-CoV-2, confluent monolayers of VERO-E6 cells were infected in triplicate with the virus at a multiplicity of infection of 0.1 in a humidified CO₂ incubator at 37°C. After 1 hour of virus-cell incubation at RT, the inoculum was discarded and cell monolayers were washed twice with PBS++. (PBS containing 100 mg/l CaCl₂ and 100 mg/l MgCl₂) and replaced with infection medium DMEM/BA [DMEM, supplemented with 0.2% BA, 1% P/S, and 1 μg/ml L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin] containing 5 μg/ml (n = 3) or without (n = 3) E/S. Supernatants were collected at 12, 24, and 36 hours after infection (p.i.) and stored at −80°C. For the titration of the viral load in the stored samples, a plaque infectivity assay was carried out as previously described (Mostafa et al., 2020; Payne, 2017).

**Protein–protein interaction (PPI) prediction**

The Prediction Server of Protein-protein InterActions (PSOPIA) prediction tool (Murakami and Mizuguchi, 2014) was used to predict the interaction between L. cuprina E/S serine protease and SARS-CoV-2 S-protein, ACE2, TMPRSS2, cathepsin B, or cathepsin L. For the study genes, STRING (Szklarczyk et al., 2019) was used to evaluate the PPI enrichment and to find the protein network of the study genes. p value < 0.05 and false discovery rate (FDR) <0.05 were considered to be significant.

**Gene expression assay**

**RNA extraction**

VERO-E6 cells were infected with 500 TCID₅₀ of SARS-CoV-2. At time intervals of 6, 12, and 24 hours after infection, cells were collected in 1 x PBS, pelleted by centrifugation, and RNA was extracted using the RNeasy Mini Kit according to the manufacturer’s instructions. The extracted RNA was treated with 2 μl (100 U) DNase I (Invitrogen) C for 1 hour at 37°C and cleaned up using 3M sodium acetate and absolute ethanol as described previously (Petersen et al., 2018). The mRNA expression of selected genes following infection with SARS-CoV-2, with and without treatment with L. cuprina E/S, was assessed via Quantitative Reverse Transcription polymerase chain reaction (qRT-PCR).

**qPCR**

Total RNAs were extracted from treated and untreated cells using the QIAamp RNA Blood Mini Kit (QIAGEN) according to the manufacturer’s instructions. The extracted mRNA was reverse-transcribed into cDNA using the RevertAid First Strand cDNA CDNA Synthesis Kit (Thermo Scientific). For qRT-PCR of Notch1, Notch2, CTNNB1, PSEN1, CDC42, BCL9, ACTR2, SUMO1, and TDG, primers were designed using the Primer3 software (https://primer3.ut.ee/,RRID:SCR_003139). Amplification mixtures were prepared using Maxima SYBR Green qPCR Master Mix (Thermo Scientific, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference gene to normalize the expression. Samples were prepared in technical replicates at three time points (6, 12, and 24 hours after infection). Results were expressed as a ratio of reference to target gene using the 2⁻ΔΔCt method (delta-delta Ct method or qPCR is a simple formula used to calculate the relative fold gene expression of samples when performing real-time polymerase chain reaction), and fold changes were normalized. Primers and annealing temperature (Tₘ) for each are listed in Table 1. qPCR cycling conditions were 95°C for 10 minutes [95°C for 15 seconds, Tₘ (as listed below in Table 1) for 30 seconds, and 72°C for 40 seconds] (40 cycles).

**Statistical analysis**

All experiments were performed in triplicate, and the data presented are as the average of the means. The significant differences (p ≤ 0.05) between the values of the control and treatment groups were determined using two-tailed independent Student’s t-tests and also two-way analysis of variances (ANOVA), followed by the Bonferroni post hoc test. The GraphPad Prism 8.02 software was used for statistical testing and graphical data display.

**RESULTS**

**Antiviral activity**

The half-maximal cytotoxic concentration (CC₅₀) of L. cuprina maggots’ E/S was measured (185.651 µg/ml) to determine

<table>
<thead>
<tr>
<th>Table 1. Primer sequences of target genes.</th>
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<tr>
<td><strong>Primer sequence (5’–3’)</strong></td>
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<tr>
<td><strong>Forward</strong></td>
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<tr>
<td>Notch1</td>
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<tr>
<td>Notch2</td>
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<tr>
<td>CTNNB1</td>
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<td>PSEN1</td>
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<td>CDC42</td>
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<td>BCL9</td>
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the appropriate concentration to identify the antiviral activity. E/S showed potential antiviral activity against SARS-CoV-2 with an $IC_{50}$ of 0.324 µg/ml and a high selectivity index (SI) value of 572.997 (Fig. 1a). The E/S could significantly reduce the growth kinetic of SARS-CoV-2 at 12, 24, and 36 hours p.i. (Fig. 1b).

**PPI prediction**

The PSOPIA prediction tool for PPI showed that *L. cuprina* maggots’ serine protease present in E/S could interact with TMPRSS2 and cathepsin B (prediction scores 0.88 and 0.95, respectively). These scores are calculated using the PSOPIA platform based on the protein sequence and 3D structure. Other proteins showed lower prediction scores.

Interactions between the studied Notch pathway genes are displayed in Fig. 2a (PPI enrichment $p$ value: 0.00189). Functional enrichments include Notch signaling involved in heart development (GO: 0061314, FDR 0.0009) and positive regulation of neuroblast proliferation (GO:0002052, FDR 0.001).

The protein network of the studied genes shows that the predicted functional partners are neural Wiskott-Aldrich syndrome protein (WASL), actin-related protein 2/3 complex subunit 3, SUMO-activating enzyme subunit 2 (UBA2), and adenomatous polyposis coli protein (cadherin-1) (prediction score 0.9999, Fig. 2b).

**Effect of SARS-CoV-2 and E/S on Notch pathway genes**

Data from RT-PCR demonstrated that SARS-CoV-2 infection resulted in a significant overexpression of Notch1, Notch2, CTNNB1, PSEN1, and CDC42 at 24 hours after infection. At time intervals 6 and 12 hours, the change in expression of all genes was insignificant compared to control. The *L. cuprina* E/S was able to significantly downregulate Notch1, Notch2, CTNNB1, PSEN1, and CDC42 in SARS-CoV-2-infected cells at time point 24 hours,
shifting their expression toward the control levels ($p < 0.0001$ except for CDC42 where $p = 0.0035$) (Fig. 3).

Also, Notch signaling downstream genes, BCL9 and ACTR2, were investigated. Consequently, SARS-CoV-2 overexpressed the BCL9 gene and underexpressed the ACTR2 gene 24 hours after infection of cells. *Lucilia cuprina* E/S significantly downregulated both genes ($p < 0.0001$). However, the relative expression of BCL9 after *L. cuprina* E/S treatment was significantly lower than its expression levels in control cells.

**Effect of SARS-CoV-2 and E/S on SUMO1 and TDG**

SARS-CoV-2 infection resulted in a significant overexpression of SUMO1 and TDG after 24 hours of SARS-CoV-2 infection. At 6 and 12 hours after infection, the change in SUMO1 expression was insignificant compared to control. TDG expression was significantly compared to control at 12 hours after infection, and no change in TDG expression was observed after 6 hours of infection.

Treatment of SARS-CoV-2-infected cells with *L. cuprina* E/S had significantly downregulated SUMO1 and TDG expressions ($p < 0.0001$) compared to untreated SARS-CoV-2-infected cells. However, the relative expression of SUMO1 after *L. cuprina* E/S treatment was still above its expression levels in control cells, while relative expression of TDG after treatment was slightly lower than its expression levels in control cells (Fig. 4).

**DISCUSSION**

The global pandemic caused by SARS-CoV-2 is currently a target of many studies aiming to prevent viral entry or disease progression. The viral S-protein is responsible for viral entry in host cells through the ACE2/TMPRESS route or the cathepsin route (*Chen et al.*, 2020). In the current study, *L. cuprina* maggots’ E/S showed potential antiviral activity against SARS-CoV-2 with a high SI value. Similar results were documented for this E/S as an antiviral agent against the Rift valley fever virus and coxsackie B4 virus (*Abdel-Samad, 2019*). One explanation for this strong effect could be the presence of serine protease in *L. cuprina* maggots’ E/S. In this study, *L. cuprina* maggots’ serine protease that presents in E/S was predicted to interact with TMPRSS2 and cathepsin B, raising the possibility that it could compete with the viral S-protein on TMPRSS2 and cathepsin B. Thus, the E/S could be effective against SARS-CoV-2 by inhibiting the two main routes of viral entry.

In the current study, SARS-CoV-2 viral load in VERO-E6 cells treated with *L. cuprina* E/S was found to be considerably low compared to nontreated cells. This could suggest that the predicted interaction of *L. cuprina* E/S with TMPRSS2 and cathepsin B could prevent viral entry.

Targeting the interaction between SARS-CoV-2 S-protein and TMPRSS2 is one of the promising approaches in preventing and treating COVID-19. In the same line, a previous study showed that blocking TMPRSS2 using several compounds could inhibit viral entry. One of the TMPRSS2 inhibitors, camostat mesylate, is an FDA-approved drug used to treat pancreatitis, with mild side effects (*Hoffmann et al.*, 2020). However, the cathepsin B endosomal pathway provides an alternative pathway for SARS-CoV-2 entry. At the same time, a previous study had shown that targeting TMPRSS2 and cathepsin B simultaneously is needed to prevent viral entry; cathepsin B inhibitors are still under trial (*Padmanabhan et al.*, 2020).

As *L. cuprina* E/S is predicted to interact with both TMPRSS2 and cathepsin B in this study and is shown to hinder viral entry, it could provide a promising agent to block the two viral entry pathways simultaneously. Yet, its efficacy needs to be assessed against the camostat mesylate and cathepsin B inhibitors.

Inhibition of viral entry is not sufficient to prevent the progression of COVID-19 infection. As the disease progresses, the viral titer titre may not correlate with the severity of symptoms, especially immune reactions and cytokine storms (*Lescure et al.*, 2020).

![Figure 3](image-url)

*Figure 3.* Expression of Notch pathway genes after 24 hours of SARS-CoV-2 infection *in vitro* (relative to control) and without *L. cuprina* E/S treatment. Notch1, Notch2, CTNNB1, PSEN1, and CDC42 were significantly downregulated upon treatment ($p < 0.0001$ except for CDC42 where $p = 0.0035$).
Hence, studying the effect of *L. cuprina* E/S on pathways that are related to immunity could provide better insight into its potential clinical usefulness.

Regarding the molecular pathogenesis of SARS-CoV-2, expression of Notch pathway genes, SUMOylation, and TDG was studied. Significant overexpression of Notch1, Notch2, CTNNB1, PSEN1, and CDC42 was observed in SARS-CoV-2 infection. Treatment with *L. cuprina* E/S significantly downregulates these genes, shifting their expression towards the control levels. The Notch pathway is one of the important pathways that play a major role in innate and adaptive immune cells differentiation and activity (Vielli Dalla Sega et al., 2019). Inhibition of Notch signaling was reported to affect immune response during viral infection and modulate the immune response to some respiratory viruses (Rizzo et al., 2020).

The Notch pathway and ACE2 and JAK/STAT pathways were reported to be dysregulated in SARS-CoV-2 pathogenesis and related to IL-6 activity (Rizzo et al., 2020). SARS-CoV-2 complications include severe inflammation and cytokine storm. Active Notch signaling has been observed under a variety of inflammatory conditions although the mechanism is not clear yet. In cytokine storm, IL-6 was reported to modulate several signalling cascades, including mitogen-activated protein kinase (MAPK) and Notch pathways (Zhang et al., 2020). Although inhibition of the Notch pathway could decrease antiviral immunity and increase viral load (Ito et al., 2011), it seems that normalization of Notch pathway genes as seen in the current study has opposite effects. The Notch pathway plays multiple roles in cell differentiation and response to infection, and evidence was documented on the role of the Notch pathway in severe COVID-19 complications such as cytokine storm, hypoxemia, and hypercoagulopathy (Breikaa and Lilly, 2021).

The ability of *L. cuprina* E/S to keep the expression of Notch 1 and Notch 2 near the control levels could suggest a mechanism by which *L. cuprina* E/S could be effective in the treatment of COVID-19. Previous reports stated that the prevention of Notch signaling pathway activation could interfere with viral entry and pathogenesis (Tursudzhyan, 2020).

Several viruses were reported to be able to evade the immune response by suppressing interferon production. The Wnt pathway was reported to play an important role in regulating immunity. Stabilization of CTNNB1 upon virus infection was proved to negatively regulate the expression of several interferon signaling genes related to innate immunity in a CTNNB1-dependent manner (Baril et al., 2013). In the current study, CTNNB1 was upregulated after SARS-CoV-2 infection, in agreement with a previous study (Vastrad et al., 2020). Lucilia cuprina E/S treatment was able to significantly decrease CTNNB1 expression, even below its levels in control cells. The reason and potential effects of this downregulation need further study.

Presenilin-1 (PSEN-1) is an endoprotease complex that hydrolyzes several transmembrane proteins (Tomita, 2017). It was reported to play a role in the Notch and Wnt signaling cascades and regulation of downstream processes via its role in processing key regulatory proteins (Thompson and Zúñiga-Pflücker, 2011). Hence, PSEN-1 is a part of the gamma-secretase complex that is critical in Notch signaling, and it is involved in the Notch-mediated inflammatory process (Zhou et al., 2015). The gamma-secretase complex is also involved in several neurological disorders (De Strooper et al., 2012). In the current study, PSEN-1 was upregulated after SARS-CoV-2 infection. Its expression levels were lower after treatment with *L. cuprina* E/S albeit still higher than the control levels. The consequences of SARS-CoV-2-mediated overexpression of PSEN-1 and its potential role in the inflammatory process of COVID-19 need further study. Also, the expression levels of PSEN-1 could help in understanding the previously reported COVID-19-related neurological disorders (Abate et al., 2020).

CDC42 was reported to be involved in the entry process of multiple RNA viruses, including coronaviruses. CDC42 has been also known to affect the cellular cytoskeleton through the formation of lamellipodia, filopodia, and stress fiber (Swaine and Dittmar, 2015). In the current study, CDC42 was overexpressed in SARS-CoV-2-infected cells, and *L. cuprina* E/S did not return reduce its levels to control levels. The importance of CDC42 in COVID-19 progression remains unclear.

Also, Notch signaling downstream genes, BCL9 and ACTR2, were studied. Herein, SARS-CoV-2 overexpressed the BCL9 gene and underepressed the ACTR2 gene 24 hours after infection of cells. Lucilia cuprina E/S significantly downregulated both genes, and relative expression of BCL9 after *L. cuprina* E/S treatment is significantly lower than its expression levels in control cells. BCL9 is known to promote CTNNB1’s transcriptional activity (Adachi et al., 2004). ACTR2 encodes for ARP2 protein that was reported to promote some respiratory viruses’ infection in lung epithelial cell lines. It was found to promote viral gene expression, protein production, viral yield, and cell-to-cell spread (Mehedi et al., 2016).

In the current study, SARS-CoV-2 infection caused significant overexpression of SUMO1 and TDG. This effect was partially prevented by the treatment of SARS-CoV-2-infected cells with *L. cuprina* E/S that had significantly downregulated SUMO1.
and TDG expression. Notably, the relative expression of TDG after treatment with *L. cuprina* E/S is lower than its expression levels in control cells.

SUMOylation of protein is known to be disrupted in viral infection. Viruses were reported to be able to manipulate the SUMOylation machinery of host cells during virus replication. Some viruses were reported to be able to activate SUMO promoters (Lowrey *et al.*, 2017). SUMO-1 was reported to be dysregulated in SARS-CoV-2 infection (Ibrahim and Ellakwa, 2021). In the current study, SUMO1 was significantly overexpressed in SARS-CoV-2-infected cells. *L. cuprina* E/S significantly shifted its levels to control levels. Inhibition of SUMO1 could be engaged in the *L. cuprina* E/S antiviral activity.

SUMOylation signaling was reported to affect the enzyme TDG which was reported to be involved in interferon response to some viral infections (Woodby *et al.*, 2018). TDG was found to be overexpressed in the current study in SARS-CoV-2-infected cells. *Lucilia cuprina* E/S decreases its levels slightly below the control levels. The SUMO1 ability to stimulate TDG (Smet-Nocca *et al.*, 2011) could provide one explanation for to the increased TDG expression after SARS-CoV-2 infection. On the other hand, increased TDG activity was shown to cause cell death through increasing double-strand breaks, and SUMOylation of TDG appears to play a protective role against cytototoxic double-strand breaks (Steinacher *et al.*, 2019). The interplay between SUMO and TDG and their role in COVID-19 need further study.

**CONCLUSION**

*Lucilia cuprina* maggots’ E/S could be promising in the prevention of SARS-CoV-2 “NRC-03-nhCoV” cell entry, potentially due to the interaction of its serine protease with host TMPRSS2 and cathepsin B. In addition, *L. cuprina* E/S minimizes the dysregulation of Notch pathway genes, SUMO1, and TDG caused by the virus. Therefore, E/S could be a potential strong inhibitor for SARS-CoV-2.

**CONFLICT OF INTEREST**

The authors state that they have no conflicts of interest.

**FUNDING**

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**ETHICAL APPROVAL**

Not applicable.

**AUTHOR CONTRIBUTIONS**

Mohammad R. K. Abdel-Samad was responsible for conceptualization, resources, methodology, investigation, writing of the original draft and its review and editing, and visualization. Fatma A. Taher contributed to resources and review and editing of the manuscript. Mahmoud Shehata and Noura M. Abo Shama were responsible for investigation. Ahmed Mostafa contributed to methodology, investigation, review and editing of the manuscript, and visualization. Mohamed A. Ali was responsible for resources. Iman H. Ibrahim contributed to resources, methodology, investigation, writing of the original draft and its review and editing, and visualization.

**DATA AVAILABILITY**

All data generated and analyzed are included within this research article.

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