International Journal of Biomedicine 13(3) (2023) 154-161
http://dx.doi.org/10.21103/Article13(3)_OA18

ORIGINAL ARTICLE

Medicinal Plants

INTERNATIONAL JOURNAL OF BIOMEDICINE

In vivo Evaluation of the Antiviral Effects of Arabian Coffee (*Coffea arabica*) and Green Tea (*Camellia sinensis*) Extracts on Influenza A Virus

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Abstract

This in vivo study was conducted to evaluate the antiviral activity of Arabian coffee (*Coffea arabica*) and green tea (*Camellia sinensis*) extracts against the influenza virus. High-performance liquid chromatography (HPLC) was used to determine the active components in each extract, and eighty experimental mice were treated. Electrophoresis was performed to detect protein expression, and reverse transcription-polymerase chain reaction (RT-PCR) was used to analyze gene expression and quantify viral RNA. Lung tissue histopathology was processed to observe pathological signs. Oral administration of all extracts reduced the viral quantification in mice lungs by 61.6% in the early phase of infection, measured by PCR. From the extracts tested, unroasted green Arabica coffee (AC) extract in protective groups showed remarkable body weight stability of 16.76 g, a survival rate of 100%, and healthier lung tissue, compared to other groups. The antiviral effects of the tested AC and GT (green tea) revealed that AC extracts induced veridical effects, increased body weight, and improved survival rate. Those natural extracts may interfere with viral replication and reduce virus infection. The observed anti-influenza activity demonstrated by reduced symptoms and increased survival rate in animal models suggests that AC extracts might be used as a promising prophylactic agent against influenza viral infections. The active compound in the unroasted green AC extract requires further in vitro analysis as to which viral proteins are targeted by the natural extract and which molecular mechanism this antiviral inhibition is interfering with.(**International Journal of Biomedicine. 2023;13(3):154-161.**)

Keywords: Influenza A virus • *Coffea arabica* • *Camellia sinensis* • RT-PCR • HPLC

For citation: Alfaifi S, Suliman R, Idriss MT, Aloufi AS, Alolayan E, Awadalla M, Aodah A, Asab OA, Al-Qahtani J, Mohmmed N, Alosaimi B. In vivo Evaluation of the Antiviral Effects of Arabian coffee (*Coffea arabica*) and Green Tea (*Camellia sinensis*) Extracts on Influenza A Virus. International Journal of Biomedicine. 2023;13(3):154-161. doi:10.21103/Article13(3)_OA18

Abbreviations

HPLC, high-performance liquid chromatography; **RT-PCR**, reverse transcription-polymerase chain reaction; **AC**, Arabica coffee; **GT**, green tea; **CGA**, chlorogenic acid

Introduction

Influenza A is a flu-causing pathogen that infects the respiratory system and has resulted in global pandemics such as the 1918 flu pandemic and the more recent 2009 swine flu pandemic.[1] The emerging variants of viruses are antiviral resistant and pose threats to existing drugs, leading to a demand for the discovery of therapeutic natural products to control and prevent viral infections.[2] Medicinal plants are the major source of novel therapeutic agents.[3] Recently, there have been numerous examples of remedies that originated from natural products,[4,5] and that have been widely used in the treatment of many diseases.[6] Many studies report that chlorogenic acid (CGA) has a wide antiviral effect against different viruses, including HIV,[7,8] adenovirus, hepatitis B virus,[9] and herpes simplex viruses,[10] and also inhibits inflammation caused by viral infection[5] and reduces serum hepatitis B virus level in vivo.[11] Numerous studies examined the effect of using GT and coffee after infection. However, they were mostly in vitro, with only a few studies in vivo.

Some medicinal plant extracts contain many beneficial constituents, such as CGA, catechins, and caffeine, that have many protective properties, such as being antioxidant, anti-inflammatory, and antimicrobial, and are important in preventing respiratory infections.[6,7,12] *Coffea arabica* and *Camellia sinensis* have a high content of CGA and caffeic acid, in common with several traditional Chinese medicines.[9,13] They are one example of natural plants with a rich source of biologically active compounds and have shown different biological effects, not only as antioxidants and anti-inflammatories, but as antifungal and anti-hyperglycemic agents.[14,15]

Green tea (GT) has organic effects and contains effective components that promote health, such as catechins, theanine, and caffeine. In previous studies, it has shown antioxidant and anti-inflammatory properties. The most significant health benefit relevant to this study is its inhibitory effect on viruses, especially influenza, by inhibiting the activity of viral RNA to prevent replication and affect the Hemagglutinin gene.[5,10] Arabica coffee (AC) extracts have effective antiviral compounds. In green, unroasted AC, there is a high content of CGA, and brown-roasted AC contains caffeine. These components have been found to inhibit RNA viruses – more sensitive than DNA viruses – in a concentration used in hot drinks.[9,16]

The chemical structure and bioactive compounds of the tea and coffee inform about the inhibitory activity on viruses. CGAs are a family of esters formed between quinic acid and cinnamic acids, such as caffeic, ferulic, and p-coumaric acids.[17,18] Of CGAs, 5′-caffeoylquinic acid is the most abundant isomer in coffee. It has been observed that CGAs influence the inhibitory activity of neuraminidase, which effectively inhibits H1N1 and H3N2 in mice.[19] Caffeine is a methylxanthine (1,3,7-trimethylxanthine) that is structurally related to adenosine and acts primarily as an adenosine receptor antagonist with psychotropic and anti-inflammatory activities.[20,21] In an in vivo study, increased doses of caffeine led to a decrease in viral protein expression and acted as a protective effect against the lethal challenge of Enterovirus A71.[22] Catechins are polyphenols that exhibit different amounts of phenolic rings attached by two or more hydroxyl groups. An in vivo study showed that mice treated with catechins enhanced CD8+ mediated adaptive immunity, reducing proinflammatory cytokine gene expression in the lungs and thus inhabiting influenza virus and SARS-CoV-1.[23,24]

Saudi Arabia was hit by outbreaks of influenza virus A-H1N1 from 2009 to 2011 and again from 2014 to 2016. This was also worsened by the MERS coronavirus epidemic in 2012.[25,14] Since coffee and tea in Saudi Arabia are consumed in high quantities daily, and different roasting procedures might result in different compounds, this study was therefore conducted to explore the effect of AC and GT extracts in drinkable forms as a natural antiviral compound against influenza A virus infection in mice.

Materials and Methods

Animal Study and Influenza A virus Infection

The study was carried out on eighty pathogen-free BALBc adult female mice provided by the animal house of King Saud University with an average weight of 17–20 g and aged from five to six weeks old. They were all housed in clean and properly ventilated cages under the same environmental conditions, with free access to food and water throughout the experiment. They were acclimatized to their environment at least two weeks before starting the experiment.

The mice were divided into three major groups (protective, infected, control) and then sub-divided into three subgroups with 10 mice in each sub-group: Group 1 was described as ‘protective pre-infection’ (Subgroup 1a included mice treated with GT extracts, Subgroup 1b had mice treated with roasted brown AC extracts, and Subgroup 1c included mice treated with unroasted green AC extracts). Group 2 was infected with the influenza virus (Subgroup 2a included mice treated with GT extracts post-infection, Subgroup 2b had mice treated with roasted brown AC extract post-infection, and Subgroup 2c included mice treated with unroasted green AC extracts post-infection). Group 3 consisted of control groups: (a. negative control group consisting of asymptomatic mice, and b. infected mice). All mice, except for the normal control group, were infected by aerosol transmission. The influenza A infection dose used in this study was 106 TCID50 (50% tissue culture infectious dose), A/Denver/1/57(H1N1). The 1.5ml of purified aerosol was delivered to individual mice in each group, with one mouse in their cage for 10 minutes. Influenza symptoms were checked daily.

Sample Preparation for Caffeine, CGA, and Catechin Determination

Three natural extracts (GT, brown AC, and green AC) were purchased from a local market. Each sample was prepared every day in a drinkable concentration. The green tea and brown

and green AC samples were first ground. About 3 g of ground samples were added to 100 ml of distilled water, placed over a heater (100 °C), and then given to the mice. Extraction was carried out for 30 minutes. Then the solution was cooled. Next, 1 ml of each extraction and 1ml of acetonitrile were added to each tube, vortexed for 1.5-minute, and then filtered through a disposable syringe filter (Chromafil Xtra PA-45/25) into HPLC vials for analysis, 10µ from each vial.[5]

High-performance liquid chromatography (HPLC)

Separation of caffeine, CGA, and catechin was carried out by HPLC (Waters Alliance 2695 HPLC Separations Module-SN: J18SM4916A-COO, the U.S.) using Xbrige waters C18 250mm×4.6mm×5.0um HPLC column at wavelength detection 210nm. The mobile phase condition was a gradient of 0.5% phosphoric acid in water mixed with methanol (70:30) v/v at a 0.55mL/min flow rate. The injection volume was 10µL; the retention time for catechins, CGA, and caffeine was 6.8 min, 7.5 min, and 8.8 min, respectively. Quantification was determined using peak area and calculated from a five-point standard curve; standard curve points were prepared by dilution in methanol.

Preparation of Stock and Working Solution

Caffeine, catechin, and CGA stock solutions were prepared by taking 1 ml of stock and 9 ml of distilled water. The working standards of the stock solution 0.1, 0.25, 0.5, 1.0, and 1.5 ml were prepared by serial dilution with methanol (Tables 1, 2, 3). The content of caffeine, catechin, and CGA of the GT, and brown and green AC samples were calculated by interpolation within the regression equation of the best line of fit. The results were then presented in mg/ml.

Histopathology Tissue Processing

Fixation and Clearing

The first step was a fixation to denature the protein to render the cells and their components resistant. It was started by using formalin for two hours containing 70% ethanol and then 80% ethanol for two hours. The next step was 90% and 95% ethanol for two hours. For clearing, we used xylene for two hours, washed it with alcohol, and then two more hours of xylene.[16]

Embedding the Tissue

After fixation, the tissue was supported by using paraffin wax to facilitate tissue cutting by using an embedding machine (Thermo Electron Corporation Shandon Histocentre SN:1293060904048, COO; the U.S.). Paraffin wax was dispensed, and the tissue was oriented in the mold, fixed to the bottom using the tamping tool, and then a cassette was placed on top of the mold and filled with wax. To solidify the paraffin wax, the tissue block was placed on a cold plate. Paraffin Section Cutting Sections were prepared by microtome (Thermo electron corporation Shandon-Finesse me+, SN: FN2076M0611, COO; the U.S.), starting with ERMA disposable Microtome Blades Patho Cutter-HP-R by trimming the surface of the block, then cutting 3–5µm thickness slices. The slices were placed in a water bath at 7 °C temperature, then slipped down the slide, removed vertically from the water, and left to dry in an oven for 10 minutes.[26]

Hematoxylin and Eosin Stain Tissue (H&E)

This step is carried out by following regressive HE stains. The slide sections are dipped into xylene baths for 5 minutes to hydrate the lung sections by decreasing the concentration of alcohol baths by 95% then 70% for 1 minute each, washed with distilled water, and then stained in Gill II hematoxylin for 3 minutes. The slide sections are then washed through running water for 2 minutes, then in distilled water and dipped in eosin for 4 minutes, then dehydrated in 95% EtOH. To remove excess eosin, slides were immersed in graded alcohols and then xylene. Once dehydration was completed, a drop of polystyrene mountant was applied, and a coverslip.[16,26]

RNA Extraction

RNA was extracted according to the manufacturer's instructions of the RNA extraction kit of QIAGEN. All centrifuge steps were carried out at room temperature. The wash buffers AW1 and AW2 were prepared; 25ml of 96%-100% ethanol was added to AW1, and 30ml of 96%-100% ethanol was added to AW2. A fresh lysis buffer AVL was prepared and added to 10µl 21 of 1-mercaptoethanol for every 1ml of lysis buffer. Tissue samples obtained from mice were caused to lyze by adding buffer AVL to motivate highly denaturing conditions, to ensure isolation of intact viral RNA, and, most importantly, to inactivate RNases. Buffer AW1 was added, followed by buffer AW2 mixed gently by the vortex. 700µl of the sample was then transferred to the spin cartridge and centrifuged at 12,000g for 15 seconds at room temperature. The used filter was discarded, then RNA was eluted in a special RNase-free buffer AVE to prevent microbial growth. Pure viral RNA was obtained and stored until used.[27]

Amplification of H1N1 Gene

Amplification was performed according to the thawing system (Applied Biosystems, Thermo Fisher Scientific Veriti 96 well thermal cycler, SN:2990234516, COO: Singapore) component and mixed by inverting several times the specific primer used to detect the presence of the H1N1 virus. Except for RNA, the following components were mixed: 25µl of SuperScript III One-Step Reaction Mix (2X), 2µl SuperScript III One-Step Enzyme Mix (25X), 2µl of Forward Primer (10µM), 2µl of Reverse Primer (10µM), 14µl of Nuclease-free H2O, and 5V of total RNA (up to 1µg), with a total volume of 50µl. The RNA template was added last, and the reactions started immediately.[28,29] We amplified two PCR products using four primers. The first product had a length of 1264bp. The second amplicon or PCR product was 913bp long. Together they overlapped and covered the entire hemagglutinin gene length of 1710 bp.

Gel Electrophoresis

The PCR products were visualized in 1% agarose gel, by mixing 1g agarose in 100ml 1×TAE buffer. The solution was boiled in a microwave for one minute. The solution was not over-boiled so that the buffer and the final percentage of agarose in the gel were not affected (Table 5). For the final concentration, 0.2-0.5µg/ml ethidium bromide was added to show the bands bound to RNA under ultraviolet light. A gel tray with a well comb was prepared at room temperature. The agarose gel without bubbles was poured into the gel tray without bubbles. Solidification was completed within 20–30 minutes. The loading buffer was then added to each sample. As it contained glycerol, it increased the density of samples to prevent diffusing. The gel box was filled with a 1×running buffer. Five microliters of 1Kb plus the DNA

ladder molecular marker and the PCR product were loaded on the gel. Gel electrophoresis was performed at 120V and 100mA for 60 minutes. Gel (Owl™ Easy Cast™ B1A Mini Gel Electrophoresis Systems, SN:300163431, COO: United States) pictures were recorded by Bio-Rad (Cheick touch Imaging Systems, SN:732BR1055, COO; Singapore).[30]

Statistical analysis was performed using the statistical software package SPSS version 26.0 (SPSS Inc, Armonk, NY: IBM Corp). The Kaplan-Meier (KM) method was used to analyze 'time-to-event' data. A probability value of $P<0.05$ was considered statistically significant.

Ethical Considerations

The proposal for research was approved by the Institutional Review Board, Faculty of Science, Princess Nourah Bint Abdulrahman University, IRB Registration number with KACST, KSA: H-01-R-059 and IRB Log Number: 19-0212. Research procedures were carried out in accordance with national and institutional regulations. Animal welfare, housing, husbandry, and pain management procedures complied with the relevant European Animal Research Association legislation.

Results

Qualification Analysis of All Extracts by HPLC

The HPLC quantification analysis method showed good separation peaks for caffeine, catechin, and CGA in different retention times (Figure 1). The quantification measurement of the components in GT, green AC, and brown AC by HPLC analysis using a PDA detector was carried out (Figure 2). Caffeine concentration has the highest value in GT rather than in green AC or AC (Table 1). CGA concentration was highest in green AC, while catechin was highest in GT.

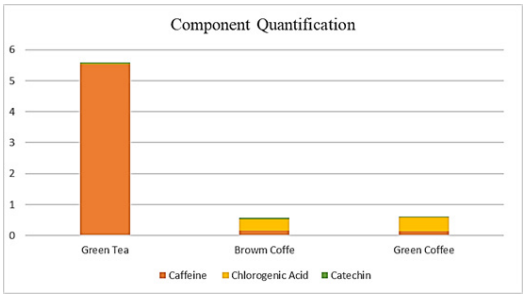

Fig. 1. *Chromatogram for active ingredient separation using HPLC.*

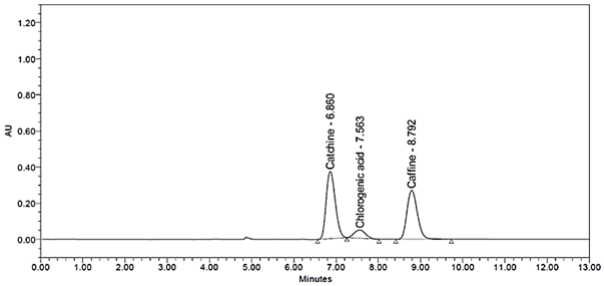

Fig. 2. *Component quantification in green tea, brown coffee, and green coffee by HPLC.*

Table 1.

Caffeine, Chlorogenic acid, and Catechin peak area and concentration of green tea, brown and green coffee.

Name of sample		Concentration (µg/mL)	Peak area	Final amount (mg/ml)
Caffeine	Green Tea	3.915	120891	0.026
	Brown Coffee	56.732	357725	0.38
	Green Coffee	66.534	420198	0.44
Chlorogenic acid	Green Tea	827.680	1954683	5.518
	Brown Coffee	23.180	525512	0.155
	Green Coffee	22.075	499020	0.147
Catechin	Green Tea	7.199	1193816	0.048
	Brown Coffee	5.196	155516	0.034
	Green Coffee	4.619	136058	0.031

Effect of Extracts on Influenza Virus

Subgroup 1c mice showed marked improved pulmonary sections manifested by fewer lesions of inflammatory cells (black arrow) and healthy lung tissue histopathology (Figures 3 a, b, and c).

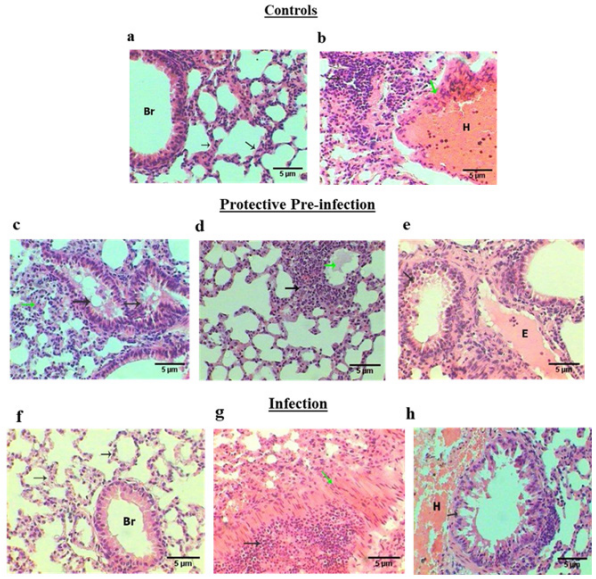

Fig. 3. *(a) Negative control lung tissue. Bronchiole (Br), alveolar intersepta (black arrows) (H&E stain: 400 X). (b) Positive control lung tissue. Hemorrhage (H), hyaline membrane (green arrow) (H&E stain: 400x). (c) Protective GC lung tissue. Inflammatory cells (black arrow), edema (green arrow) (H&E stain: 400x). (d) Protective GT lung tissue. Hyperplasia (black arrow), edema (E) (H&E stain: 400x). (e) Protective BC lung tissue. Collapsed bronchioles (black arrows), leukocytic exudate (green arrow) (H&E stain: 400x). (f) Treated with GT lung tissue. Bronchiole (Br), alveolar intersepta (black arrows) (H&E stain: 400x). (g) Treated with BC lung tissue. Inflammation (black arrow), hyaline membrane (green arrow) (H&E stain: 400x). (h) Treated with GC lung tissue. Hemorrhage (H) destructed bronchiole cells (black arrow) (H&E stain: 400x).*

In addition to steady body weight, there was a slight rise in lung weight and a stable survival rate (100%) compared to the positive and negative controls (Table 2). Subgroup 1b mice displayed some pathological changes represented by hyperplasia (black arrow) of bronchioles columnar epithelia, in addition to the formation of hyaline membranes and edema (Figure 3 (e). There was steady body weight, a slight rise in lung weight, and a small decrease in survival rate (80%) compared to the positive and negative controls. Subgroup 1a mice showed marked pathological alterations characterized by collapsed bronchioles (black arrow) filled with edema, as well as exudate of leukocytic infiltration (green arrow) (Figure 3 (d). Steady body weight, a slight rise in lung weight, and a stable survival rate (100%) were observed when compared with the positive and negative controls. Polyphenolic compounds present in all these extracts, particularly catechin, and CGA, are known to have strong anti-influenza activity by inhibiting various steps in the virus life cycle. Thus, unroasted green AC extracts showed the strongest antiviral activity, compared with roasted brown AC and GT.

Table 2.

The measurement of body weight and lung weight in grams and the survival rate (%) of each group.

Group	Subgroup	Body weight (g)	Lung weight (g)	Survival rate (%)
Protective group	1a) green tea	16.76	0.30	100
	1b) brown coffee	15.70	0.36	80
	1c) green coffee	16.73	0.33	100
Control group	a.) negative control	16.31	0.34	90
	b.) positive control	12.21	0.41	30
Infected group	2a) green tea	13.88	0.34	40
	2b) brown coffee	15.94	0.41	80
	2c) green coffee	16.27	0.37	80

Subgroup 2c mice showed improvement, manifested by completely opened bronchioles and thin healthy alveolar septa (black arrow) (Figure 3 (h). However, Subgroup 2b mice showed severe alterations represented by thick alveolar septa due to the exudate of inflammatory cells, the formation of wide hyaline membranes, and great aggregations of inflammatory cells (black arrow) (Figure 3 (g). Furthermore, Subgroup 2a mice revealed adverse effects manifested by the destruction of the bronchiolar columnar epithelia (black arrow), in addition to wide areas of hemorrhage (Figure 3 (f).

Experimental Use of Mice

Protective pre-infection subgroups were treated for two weeks before the infection with GT, brown AC, and green AC extracts. These mice were healthy and hyper-activated after the infection. The bodies of the mice were weighed daily. Lung weight was taken on day seven after infection initiation (Table 2). We noticed from Days 2–5 that the mice experienced slight weight loss, hair redness, lack of energy, and eye and nose irritation (Figure 4 (a).

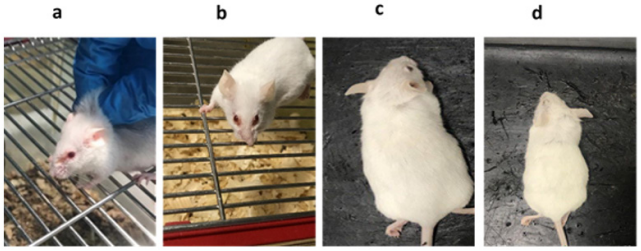

Fig. 4. *Experimental Animals*: **(a)** *Mice infected with H1N1-protective group.* **(b)** *Mice infected with H1N1-infected group.* **(c)** *Negative control-healthy mouse.* **(d)** *Positive control infected with H1N1.*

In infected subgroups, symptoms appeared within 2-5 days: significant weight loss, hair redness, and eye and nose irritation (Figure 4 (b). In addition, there was an energy reduction, and the number of deaths increased sharply in infected groups. The mice were weighed daily, and lung weight was taken on Day 7 after infection initiation (Table 2).

PCR and Gel Electrophoresis

In addition to the results of lung tissue observations, survival rate analysis, and clinical observations, PCR products were not detected in Group 1. Supplementary Figure 1 shows negative PCR results for the H1N1 hemagglutinin (HA) gene analyzed for Subgroups 1a-c. For gel band quantification, we further analyzed the thickness of each band in Group 2 using ImageJ software (Table 3). The extracts showed the potent inhibition effect of influenza virus replication in the lungs of infected mice.

The HA gene for H1N1 influenza viruses with expected PCR product (1264 bp) for Subgroups 1a-c and HA gene for

Table 3.

Quantifying Hemagglutinin gene (HA) bands in infected groups showing the thickness of each band by using ImageJ software.

Quantification of the *HA* gene bands								
	Green Tea	Brown Coffee	Green Coffee	Green Tea	Brown coffee	Green Coffee	Positive Control	Negative Control
ImageJ quantification	395055	232472	212829	212829	345785	337908	881860	128180

H1N1 influenza viruses with PCR product (1264 bp) for Subgroups 2a-c (Figure 1). In Group 2, we observed flu-like clinical signs. Following this observation, extracts were given, and weight loss, respiratory symptoms, and survival rates were recorded. In agreement with findings from the lung tissue when observing inflammatory cells, PCR results also showed bands HA (1264 bp) representing the effect of treating mice with the extract after the onset of clinical symptoms (Figure 1).

Discussion

In this study, we reported that HPLC quantification analysis showed the highest caffeine catechin contest was in GT; these findings are in agreement with a study by Lee et al.[31] that found GT inhibited the influenza A-H1N1 in chicken egg and mice models but did not reduce the symptoms. The study addressed the effect of GT as an antiviral, with evidence of inhibiting the influenza virus A H1N1 in the early phase of the virus's life cycle. Supporting the current study, in the Subgroup 1a mice with protective pre-infection GT use, GT had the highest caffeine and catechin, whereas, in a study by Lee et al.,[31] GT catechin inhibited initial entry of the influenza virus.

Our findings suggest an association between treatment in the protective pre-infection Subgroups 1a-c and stable body weight and a decrease in symptoms, as histopathology showed healthy lung tissue with a few inflammatory cells in protective groups. However, treatment after infection did not present this association. Using quantitative reverse transcription PCR, Lee with colleagues[31] found inhibitory effects in neuraminidase and strong inhibitory activity on hemagglutinin in the protective pre-infection GT group.

In 2014, Utsunomyia et al.[32] studied the effect of reagents caffeic acid, quinic acid, and CGA on the multiplication of the influenza A virus. They found that the infected cells were suppressed after receiving the reagents in the early stage of the infection. The degree of suppression became less prominent with the delay of the reagent to the infected cell culture. These results help to explain the results from the current study, as the protective pre-infection subgroups presented with a healthier body and lung weight, a higher survival rate, decreased inflammation of cells in lung tissue, and negative bands in gel electrophoresis of the virus in the lungs and infected groups experienced a decrease in body weight and an increase in lung weight. The relationship between lung tissue weight before and after treatment showed an increase in infected lung weight due to an increased number of inflamed cells and serous fluid. Histopathology showed apparent inflammation in lung tissue and positive clear bands in gel electrophoresis.

These findings are likely to be due to the coffee components strongly interfering with the multiplication of the influenza A virus. When added in the early stage of infection, coffee extract interacts with certain enzymes necessary for preparing viral RNA and affects the formation of progeny virus to a limited extent when added after the onset of RNA replication.[32] The reagents that used Utsunomyia with colleagues [32] were chemically modified, not natural extracts with multiple effective components. There was no in vivo experiment in their study to test the effect of these reagents on live infections and the subsequent effects on health. CGA seems to have the ability to reduce significant symptoms and increase the survival rate.

Most research involving natural extraction illustrates an antiviral effect in different proteins in the structure of the virus. Components also acted as inhibitors in the first stage of the life cycle of the virus. However, similar studies have examined the antiviral effect after infection; most have been in vitro, and only a few in vivo.[33] Our findings demonstrate that natural extraction plays an important role as an anti-influenza with minimal side effects, most likely due to the inhibitory effects of catechins, which could be interpreted as suppressed viral replication activity. The replication is thus halted by preventing the release of the virus.[34] CGA on NS1 (nonstructural protein 1) is a protective viral protein that regulates the host gene expression and disarms interferon.[35]

This research concluded that natural extraction has an important and effective role in reducing the symptoms of infections and increasing survival rates. The results show that GT is the most effective, reducing both the viral quantity in the lungs and pathological signs in histology. GT tea showed a strong effect on survival rates and reduced symptoms, especially in the protective groups. Despite these promising results of correlations between natural extraction, reduced symptoms of the influenza virus, and increased survival rate with minimal side effects, questions remain unanswered as to which viral proteins are targeted by natural extract and which molecular mechanisms the antiviral inhibition is interfering with. More studies need to address different concentrations of natural extracts and compare them with active molecules using GC-Ms spectrometry.

The study highlighted the protective effect of green tea and Arabica coffee on the influenza virus in vivo. To better understand the implication of these results, future studies should focus on the impact of green tea and Arabica coffee on influenza viruses and other respiratory viruses in vitro, addressing the effects on the virus life-cycle in order to determine the direct medicinal impacts of natural extraction on the structure of viruses or indirectly on the immune system.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

The authors acknowledge the assistance provided by the Infectious Diseases Vaccines Chair at King Saud University and King Abdulaziz City for Science and Technology, Life Science & Environment Research Institute labs. The authors are also thankful for the training provided by Mr. Saad Alamri and Mr. Murad Alshehry at King Fahd Medical City.

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