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Evaluation the Antiviral Actvity of the Sweetened Kombucha Tea Against Various Viral Infections

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Abstract

The current study examined at the in vitro inhibitory effects of fermented kombucha tea on hepatitis A virus, rift valley fever virus, and herpes simplex type 1. Our findings indicated variable degrees of antiviral activity against three viruses with different levels of selective indices (SI = 11.19, 4.95, and 2.03, respectively). During the virucidal phase, Kombucha tea was effective against hepatitis A virus and Rift Valley fever virus, but had no impact on herpes simplex virus type-1, according to the mechanism of action assay.

Keywords: Kombucha tea; antiviral activity; herpes simplex virus; hepatitis A virus; rift valley fever virus

1. Introduction

The current COVID-19 virus epidemic necessitates a high need for non-conventional antiviral medicines that can lower infection risk and facilitate rapid recovery. Fermented foods have lately gained popularity due to their supposed strong antiviral activity. Fermented foods can be found in traditional cuisines worldwide. Their diversity is mostly dictated by people's dietary preferences as well as the availability of raw materials [1]. The Asian, Middle Eastern, and North African regions have a wide variety of fermented foods made from plant or dairy-based raw materials, such as soybeans for tempeh, cabbage for kimchi [2], durian for tempoyak [3] and glutinous rice tapai [4], sorghum and maize for fermented porridges [5], and sugared tea for kombucha [6]. Kombucha, a traditional drink that originated in Northeast China and later spread to East Russia, is extensively used as a medicinal health-promoting beverage across the world [7]. Kombucha is also known as haipao, teakwass, tea fungus, Manchurian mushroom, and kambotscha. It is made by fermenting sugared black tea with an acetobacter and yeast culture. Kombucha fermentation is primarily driven by lactic acid bacteria such as *Lactobacillus* and *Leuconostoc*, as well as acetic acid bacteria, *Acetobacter*, and *Gluconobacter*, and yeasts such as *Zygosaccharomyces* spp., *Saccharomyces* spp., and *Brettanomyces* spp [8]. As a by product of ethanol production, yeasts hydrolyze sucrose to glucose and fructose, whereas acetic acid bacteria convert ethanol and glucose to acetic acid and gluconic acid, respectively [9]. Other metabolites produced by kombucha fermentation include lactic, citric, malic, and glucuronic acids, as well as vitamins, minerals, and phenolic compounds. Traditional kombucha fermentation substrates are black or green tea; but, in recent years, there has been a movement toward the use of alternate carbon and nitrogen sources, with the goal of boosting the functionality of the final beverage. Kombucha's success has been ascribed in part to its supposed health advantages, which include strengthening the immune system, easing IBS symptoms, assisting in weight reduction, and lowering blood pressure, to mention a few. However, the majorities of these claims are unproven and lack scientific support, and there have been few clinical studies conducted to date. Furthermore, there have been instances of toxicity related with excessive kombucha drinking, particularly in immunocompromised patients [10]. On the other hand, antimicrobial effects against Gram-positive and Gram-negative bacteria such as

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Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Klebsiella pneumoniae have been observed in several reports [10-13]. Kombucha's pH ranges from 2.5 to 4.6, making it acidic enough to battle viral diseases such as the Foot-and-mouth disease virus [14, 15]. However, no other studies have shown that kombucha has an antiviral impact. Therefore, the present study describes a series of experiments conducted to better characterize the antiviral activity of kombucha against hepatitis A virus (HAV), rift valley fever virus (RVFV), and herpes simplex virus type-1 (HSV-1).

2. Materials and methods

2.1. Cells and viruses

African green monkey kidney (Vero) and hepatocellular carcinoma (HEPG-2), (ATCC, USA) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, GIBCO, USA) supplemented with 10% fetal bovine serum (FBS). In a humidified incubator with 5% CO_2, the cultures were incubated at 37°C. Holding Company for Biological Products and Vaccines (VACSERA), Egypt generously contributed HAV (H-10), RVFV, and HSV-1 for *in vitro* viral challenge. The viral titers were calculated using the limit-dilution method and were expressed as a 50% cell culture infective dosage of 1 X $10^{6.5}$ (HAV), 1 X $10^{7.5}$ (RVFV), and 1 X 10^{6} (HSV-1). Virus stocks were kept at 80°C until they were used.

2.2. Preparation of the kombucha tea

Prof. Dr. Khaled Dougdoug, Faculty of Agriculture, Ain Shams University, Egypt, kindly contributed a Kombucha culture consisting of Saccharomyces pastonanus and Acetobacter xylinum. In one liter of distilled water, combine 50 g of sugar, 1 g of $(NH_4)_2SO_4$ and 1 g KH_2PO_4, 5 g of licorice, 2 g of grosvenor momordica, 2 g of chrysanthemum, and 2 g of green tea. After boiling for 15 minutes in a sterile conical flask, the solution was allowed to cool at ambient temperature. The cooled solution was sterilized using a 0.22µ filter before being seeded into Kombucha culture for fermentation. Fermentation was carried out under aseptic conditions, and recovered metabolites were filtered via 0.22µ filters (Millipore-USA). Finally, to guarantee the sterility of the metabolites, agar, thioglugolate, and soya bean broth culture mediums were utilized [15-17].

2.3. Determine the cytotoxicity using MTT assay

According to Allayeh et al. [18] and AbouAitah et al. [19], incubated 100µl of 2x10^4 cells/well in 96-well plate for 24 hours. The growth medium was removed, and various amounts of filtrate (1000, 500, 250, 125, and 0 µg/ml) were inoculated in fresh medium for another 72 hours at 37°C in a humidified atmosphere of 5% CO_2. The medium was replaced with 100 µl of MTT solution (5 mg/ml) and incubated for 4 hours at 37°C. The MTT solution was replaced with 50µl of acidified isopropanol after 30 minutes at 37°C. A plate reader was used to determine the optical density at 570 nm. At 570 nm, the optical density was determined using a plate reader. The 50% cytotoxic concentration (CC50) was estimated as (AB/A) 100, where A & B are the means of three OD570 measurements of untreated and treated cells, respectively.

2.4. Efficacy of kombucha tea against viral infections

Vero and HepG-2 confluent 96-well plats were infected for 60 minutes at 37°C with 100 µl of stock HAV, RVFV, and HSV-1 viruses. The filtrate was then added in 100 µl increments. Three wells were utilized for each dilution, and 100 µl of the maintenance medium was added to each well. Plats were finally incubated for three days until complete CPE was observed. The antiviral activity relative to control was used to assess CPE inhibition, which was expressed using the Reed and Munech formula [20].

2.5. Mechanisms of the inhibition activity

Plaque reduction and CPE inhibition assays were used against three viruses as previously described [21-23] to assess if the candidate compounds have a "virucidal impact" on the viral particle and/or interfere with viral adsorption and/or viral reproduction during the virus life cycle.

- Mechanism of Viral Adsorption

  Vero cells (for HSV-1 and RVFV propagation) and HepG-2, (for HAV propagation), (10^5 cells/mL) were cultured in a 6-well and 96 well plates, respectively for 24 hours at 37°C to test the viral adsorption process. Compounds were co-incubated with cells in media at 4°C without supplementation. Three washes with supplement-free media were used to eliminate the non-absorbed compounds. Pretreated cells were co-incubated for 1 hour in DMEM media with diluted viruses and 2% agarose. Plates were hardened and incubated at 37°C until viral plaques or CPE formed. For 1 hour, cells were fixed in 10% formalin solution and stained with crystal violet. In contrast to control wells, the relative percentage of plaque development decrease was determined.

- Viral Replication Mechanism

Cells sown in a 6-well and 96 plates at a concentration of (10^5 cell/ml) were infected for 1 hour with virus after a 24-hour incubation period at 37°C. The non-absorbed virus particles were cleaned three times with supplement-free media. Candidate compounds were given to infected cells at various doses for another 1 hour. The inoculum was withdrawn and replenished with DMEM containing 2% agarose. then allowed to firm until viral plaques or CPE formed. Following that, plaques and CPE were fixed, stained, and computed as previously stated.

- Virucidal Mechanism

In a 6-well and 96 well plates, cells were seeded at a concentration of (10^5 cells/mL) for 24 hours at 37°C. Following that, serum-free DMEM containing each virus was introduced to each compound sample. After 1 hour of incubation, this combination was diluted two-fold three times with serum-free media. Following that, 100 µl of each dilution was applied to the cell monolayer. After 1 hour of interaction with the cells, medium was introduced. plates were allowed to firm until viral plaques or CPE formed. The plaques were fixed, stained, and computed as previously reported.

2.6. Statistical analysis

All experiments were performed in triplicate and calculations were carried out using GraphPad PRISM and linear regression analysis (Version 8.0.1, GraphPad Software, San Diego, CA, USA). The selective index (SI) was derived using CC50/IC50.

3. Results

In the recent decade, there has been a surge in the number of scientific papers on the biological activity of fermented kombucha tea. Despite several in vitro studies on kombucha's antibacterial activity, only two antiviral trials against FMD virus have been published. The present study will examine at how fermented kombucha tea suppresses Hepatitis A virus (HAV), Rift Valley fever virus (RVFV), and Herpes simplex virus type-1 in vitro.

3.1. Efficacy of the cytotoxicity of the kombucha tea

The cell viability of Vero and HepG-2 cell lines was assessed using the MTT test after 72 hours of incubation. The 50% cytotoxic and growth inhibitory doses of kombucha tea were calculated using the mean dose-response curves of three different trials. The fermented Kombucha tea was not toxic to either cell line at higher concentrations and the maximum non-cytotoxic concentration was determined to be 77.66 µM on Vero and 242.8 µM on HepG-2 cell lines (Fig. 1).

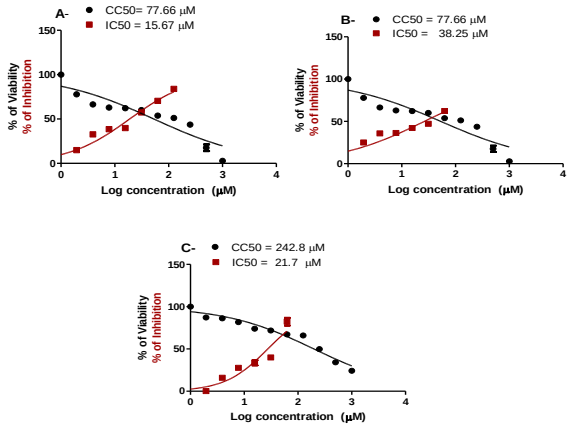

Fig. 1 The cytotoxicity (CC50) and the inhibitory concentration (IC50) of sweetened kombucha tea on Vero and HepG-2 cell lines infected by various viruses. A) RVFV on Vero cell line; B) HSV-1 on Vero cell line; and C) HAV on HepG-2 cell line. Data is shown as mean ± SD; (n = 3).

3.2. Efficacy of the Antiviral Activity

Antiviral activity of kombucha tea against HAV, RVFV, and HSV-1 was determined in vitro. The assay used untreated virus-infected cells as a control. The 50% inhibitory concentration (IC50) for HAV, RVFV, and HSV-1, respectively, was reported to be 21.7, 15.67, and 38.21 µM. The results are shown in (Table 1) as a selective index (SI) of 11.19, 4.95, and 2.03, the average of three distinct tests. With a selectivity score of 11.7, the kombucha tea successfully reduced the human hepatitis A virus, making it a viable antiviral treatment against HAV. While the kombucha filtrate exhibits low selectivity indices of 4.95 and 2.03 for RVFV and HSV-1, respectively, it is a weak inhibitor for both viruses. HAV's infectivity was lowered by more than 69% at peak non-cytotoxic concentrations of the kombucha filtrate, followed by 25% for RVFV and 12.5% for HSV-1.

Table 1 Inhibitory concentrations and selective index for kombucha tea against various viruses

Sample	Virus	IC_{50} (µM)	SI
Sweetened kombucha tea filtrate	HAV	21.7 ± 0.91	11.19
	RVFV	15.67 ± 0.58	4.95
	HSV-1	38.21 ± 1.34	2.03

3.3. Mode of action for HSV-1 and RVFV infections

A plaque reduction experiment for HSV-1 and RVFV was performed to determine if fermented kombucha tea directly interferes with viral replication by having a virucidal effect, or indirectly by blocking viral adsorption into host cell receptors or impeding intracellular viral reproduction. RVFV is suppressed by 39.5% after fermented kombucha tea is administered. Furthermore, interfering with viral adsorption resulted in a significant viral drop (19.7%), but had no influence on RVFV virus reproduction. Fermented kombucha tea exhibited no effect on Herpes simplex virus during the virucidal, adsorption, and replication stages (Fig. 2).

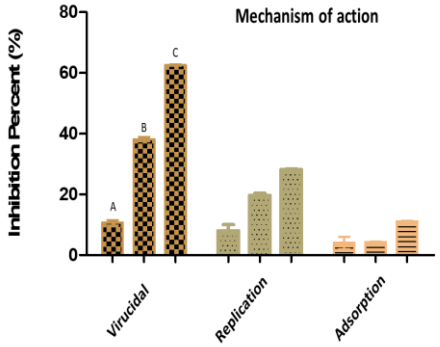

Fig. 2 Mode of inhibitory mechanism for sweetened kombucha tea against (A) HSV-1, (B) RVFV and (C) HAV viruses. By using the plaque reduction or CPE inhibition assays, the three principal mechanisms were evaluated: virucidal, adsorption, and replication. Data is shown as mean ± SD; (n = 3).

3.4. Mode of action for HAV infection

As previously mentioned, a CPE inhibition experiment for the HAV virus was carried out to assess if fermented kombucha tea interferes with viral replication directly or indirectly. The fermented kombucha tea has a virucidal impact on HAV, reducing it by over 72%. Furthermore, interfering with viral adsorption resulted in a significant viral drop (28.2%), but had no influence on HAV virus multiplication (Fig. 2).

4. Discussion

Certain herbal supplements have been found to have antiviral efficacy across the board. Chinese kombucha tea is made by fermenting Chinese herbal extracts with a kombucha culture. Previous research has demonstrated that this type of herbal kombucha tea efficiently reduces FMDV proliferation in vitro and in vivo [15, 24]. The present study will examine at the antiviral activities of fermented kombucha tea against the viruses HAV, HSV-1, and RVFV. Initially, fermented Kombucha tea was not harmful to Vero and HepG-2 cell lines at higher concentrations; these findings are consistent with those of recent report by Fu et al. [15], who investigated whether the organic acid content of kombucha therapy contributes to some of the protective benefits shown in vitro. Furthermore, the findings are consistent with Ziska et al. [25], who found that kombucha tea may be utilized to improve the cytotoxicity of Solanum nigrum extract against the MCF-7 breast cancer cell line.

The 50% inhibitory concentration (IC50) for HAV, RVFV, and HSV-1, respectively, was reported to be 1.049, 2.016, and 6.254 µg/ml. The kombucha tea successfully reduced the human hepatitis A virus, making it a viable antiviral treatment against HAV. While the kombucha filtrate exhibits low selectivity indices for RVFV and HSV-1, respectively, it is a weak inhibitor for both viruses. Based on the differences in the findings found in this study with different virus types, it appears that fermented kombucha is more efficient than DNA viruses (e.g., HSV-1) at preventing RNA viral infections (e.g., HAV and RVFV). These findings support the indicated conclusion of the sole earlier publication on kombucha's antiviral activity, which revealed kombucha's capacity to inactivate FMDV (RNA virus) in vitro [15]. Surprisingly, fermented kombucha tea suppressed HAV and RVFV with strong to moderate viral reduction, respectively. Furthermore, interfering with viral adsorption resulted in a significant viral reduction but had no effect on HAV and RVFV reproduction. Fermented kombucha tea showed no impact on the Herpes simplex virus. When this compound was delivered to host cells before or after infection, however, it had no effect. These findings are consistent with previous studies, which found a significant direct inactivation of FMDV free viral particles [15, 24].

5. Conclusions

The present study demonstrated that kombucha tea may be exploited as a helpful source of natural acids, vitamins, polyphenols, and alkaloids with anti-viral properties, notably against the HAV and RVFV viruses.

6. Abbreviations

HAV, hepatitis A virus; HSV-1, herpes simplex virus type 1, RVFV, rift valley fever virus; CC50, 50% of cytotoxic concentration of the tested compound; IC50, 50 % of inhibitory concentration.

7. Conflicts of interest

The authors declare no conflict of interest

8. Funding sources

This research received no funding

9. Acknowledgments

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10. References

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