The impact of filtered water-pipe smoke on healthy versus cancer cells and their neurodegenerative role on AMPA receptor

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ABSTRACT
Water pipe smoking is highly prevalent in developing countries, especially in Eastern Mediterranean regions. Research finds that more than 100 million people smoke a water pipe. Furthermore, tobacco smoking is one of the leading behavioral factors related to an increased risk of cancer, a leading cause of death globally. We aim to introduce a novel filtration system for water-pipe smoking and evaluate cytotoxic effects of common water pipe condensed smoke in comparison with our novel filtration system on normal (HEK293t) and cancer cell lines (Hep3B and MCF7) by MTS assay, alpha-fetoprotein (aFP), and apoptosis/necrosis effects. More so, the smoke substituents’ neurotoxicity effect was evaluated by analyzing the depressive property on AMPA receptors (AMPARs). Our results showed that the silica filtration system was more effective than the water filtration system. The number of toxic compounds was reduced from 145 mg in distilled water extract (DWE) to 57.5 mg in silica solution extract (SSE). The SSE method also showed lower toxicity impacts on normal and cancerous cell lines (HEK293t, Hep3B, and MCF7) with CC50 values 149.9, 10.14, and 8.9 μg/ml, relative to the DWE method (CC50 values 77.1, 3.1, and 5.24 μg/ml, respectively). SSE extraction also reduced the α-FP (tumor marker test) to 2273.3 ng/ml which was closer in value to untreated cells (4066.7 ng/ml) in comparison with DWE which reduced it greatly to 1658.7 ng/ml, and the biophysical properties of AMPAR subunits demonstrated a reduced effect on desensitization rates of GluA2 homomer and GluA1/2 heteromer, using SSE relative to DWE. In conclusion, the condensed smoke of ordinary water pipe (DWE) has cytotoxic and neurotoxic impacts on various cell lines, while our newly developed system (SSE) was less toxic.

INTRODUCTION
Tobacco smoking, in its various forms, is one of the leading behavioral factors related to the increased risk of cancer, and one of the leading causes of death globally (Azab et al. 2018, Hawash MM and Baytas 2018). Cigarette smoking is a health-threatening factor associated with severe diseases such as hypertension and atherosclerosis (Virdis et al. 2010). It has been found that tobacco kills around six million people annually, most of which live in low-income countries, yet the number of deaths is expected to reach 8.3 million by 2030 (Husain et al. 2016). More specifically, shisha smoking, a popular form of tobacco smoking, has been associated with the development of different cancer types such as Keratoacanthoma and lung cancer (El-Hakim and Uthman 1999, Al-Belasy 2004). Findings also suggest that shisha smoking is associated with other deleterious health outcomes including periodontal disease respiratory illness, low birth weight, and the development of heart diseases like blood pressure, tachycardia and right ventricular function deterioration (Mazen and Aurabia 2000, Al-Belasy 2004). More so, shisha smokers are found to have a significant decrease in lung function parameters (Meo et al. 2014).

Water-pipe usage is a 600-year-old practice known to different regions as nargileh, hookah, hubbly-bubblie, or argileh (Aanyu et al. 2019). It is a unique smoking method in which the smoke moves through water before its inhalation (Aslam et al. 2014). It is considered an attractive and popular smoking style, especially among females and youths, and seems to be replacing cigarette use in certain regions (Khayatzadeh-Mahani et al. 2017). The water pipe consists of three main parts (Figure 1). The head (bowl) sets on a head gasket and holds some flavored tobacco covered by perforated aluminum foil. Hot coal pieces are placed atop the foil, heating the tobacco in the bowl and initiating the release of smoke. The smoke then travels through a stem or shank that connects the head to a water-filled vase, and finally is inhaled through a hose linked to the vase. As the smoker pulls a breath in, heat transfer from the coals to the tobacco accelerates and allows for the generated smoke to get drawn through the stem into the vase, where it rises above the water and into the hose (Akhter et al. 2014).

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Since the mid-1990s, water pipe smoking has become an epidemic around the globe with more than 100 million people worldwide smoking a water-pipe daily (Al-Naggar et al. 2014). Moreover, there has been a noticeable increase in the number of water-pipe cafes. In the UK, for example, there has been a 210% increase in 2007 alone (Kadhum et al. 2014). Research shows that water-pipe users inhale around 200 times more smoke in a single session than cigarette smokers do (Anjum et al. 2008). On the other hand, contradictory findings show that the water in water pipes acts as a highly efficient filter in its absorption of ~91% of tar from the heated tobacco, and an effective heating medium for the drug, supporting the notion that water-pipe smoking is less toxic than cigarettes (Chaouachi 2009). A 2019 cross-sectional study was carried out on university students in the Eastern Mediterranean Region (Egypt, Jordan, and Palestine) with results showing that 63.1% of the Palestinian participants alone participate in this behavior (Salloum et al. 2019). Shisha smoking, or waterpipe smoking, popularity is believed to be influenced by multiple factors, such as social and cultural beliefs, low cost and easy accessibility, interesting designs and assemblies, and the availability of various tobacco flavors (Muassel) (Amin et al. 2010). Muassel constitutes 30% tobacco and 70% honey, humectants, and a fruit taste such as apple, mint, grape, watermelon (Kotecha et al. 2016). The exacerbated use of shisha use also lies in the misunderstanding that the nicotine content in water-pipe smoking is less toxic than cigarettes (Chaouachi 2009). A 2019 cross-sectional study was carried out on university students in the Eastern Mediterranean Region (Egypt, Jordan, and Palestine) with results showing that 63.1% of the Palestinian participants alone participate in this behavior (Salloum et al. 2019). Shisha smoking, or waterpipe smoking, popularity is believed to be influenced by multiple factors, such as social and cultural beliefs, low cost and easy accessibility, interesting designs and assemblies, and the availability of various tobacco flavors (Muassel) (Amin et al. 2010). Muassel constitutes 30% tobacco and 70% honey, humectants, and a fruit taste such as apple, mint, grape, watermelon (Kotecha et al. 2016). The exacerbated use of shisha use also lies in the misunderstanding that the nicotine content in water-pipe smoking is less than in cigarettes, and that water filters out toxins present in the Muassel, including nicotine, tar, and carbon monoxide (Anjum et al. 2008). A new form of water-pipe smoking, water pipe-pen, has recently become popular and is also available in different flavors (similarly to the traditional shisha) and may come with or without nicotine. This water-pipe pen mimics the electronic cigarette that has been introduced in the last couple of years. However, despite its promised healthier potential, the pen’s main components are propylene glycol and glycerin, found in high concentrations, and cause airway irritation (Kienhuis et al. 2015). 

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), a subtype of glutamate receptors, are fast-firing ionotropic receptors and essential in the genetic coding for nervous system circuitry development in the human brain and processes involved in memory and learning (Zhao et al. 2019). These receptors are composed of four subunits, earning their tetrameric structure identity (Robert and Howe 2003). Their subunits are GluA1, GluA2, GluA3, and GluA4, and the receptors can be assembled either into a homomeric structure with four same subunit types, or heteromeric combinations of two (or three) subunit types. The most prevalent AMPAR assemblies found in the brain are GluA2 homomeric tetramer, followed by the heteromeric GluA1/2 assembly (Zhao et al. 2019). A reduction or lack of GluA2-type receptors in neurons has been associated with the development of human brain disease and abnormal function, including Alzheimer’s disease and epilepsy (Qneibi et al. 2019). More so, reduced receptor numbers lead to an accumulation of Calcium concentrations in postsynaptic neuronal membranes, allowing for the progression of these detrimental consequences (Zhao et al. 2019). AMPARs are activated by glutamate binding, opening their channels by causing conformational changes in the receptor assembly. Similarly, AMPAR signaling can be dampened through deactivation or desensitization mechanisms by binding to competitive and noncompetitive inhibitors, which act to close the channel pores and prevent postsynaptic signal pathway activation (Coombs et al. 2019).

The literature showed that gas chromatography-mass spectrometry (GC-MS) is primarily used in determining and extracting toxins of water-pipe smoke, such as 1-naphthylamine, 2-aminobiphenyl, 2-naphthylamine, 2-furaldehyde, 2-furoic acid, 2-furyl methyl ketone, 3,5-dichloroaniline,
4,4′-oxydianiline, p-chloroaniline, 5-methyl-2-furaldehyde, aniline, carbon monoxide, furfuryl alcohol, and 5-(hydroxy-methyl)-2-furaldehyde (Shihadeh et al. 2015, Middha and Negi 2019). Our findings showed that no previous studies have tried to condense the smoke generated from the water pipe or used liquid chromatography-mass spectrometry (LC-MS) to detect the toxins that might accumulate in a smoker’s lungs after inhaling the emitted smoke. Therefore, in this study, we aim to detect new toxins from the water pipe through the aforementioned technique, define a new filtration system, and decrease the toxic compounds’ concentrations to reduce their cytotoxic and neurotoxic effects.

Methods and materials

All of the chemicals and reagents used were purchased from reliable resources Sigma Aldrich, ATCC. The following chemicals were used throughout this study: methanol, ethyl acetate, sodium bicarbonate, silica, DMSO 0.1%, and Doxorubicin (Positive control). The following materials were purchased form the Palestinian market: Muassel ‘double apple’, tea, milk, sugar, and coal. The following instruments were used: Thin Layer Chromatography (TLC), UV light, Rota-evaporator, vacuum generator, SHISHA and aluminum foil. High-resolution mass spectral data (HRMS) were collected using a Waters LCT Premier XE Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) using ESI (þ) method. The instrument is coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA, USA). These tests were done at Pharmacy Faculty Gazi University Ankara-Turkey.

Chemical methods

Preparation of filtration fluids

Distilled water (DWE) and milk were used as such. Sodium bicarbonate (NaHCO₃) was prepared by dissolving 2 g in up to 200 ml of distilled water (DW). Tea solution (200 ml) (TSE) was prepared by steeping 2.04 g of tea in up to 200 ml of hot (DW) then cooling to room temperature. The sirup solution was prepared by dissolving 2 g of sugar in up to 200 ml of DW. Two concentrations of silica solution (SSE) were prepared by steeping 50 g or 10 g of silica in up to 200 ml of DW. In each experiment, one of the above-mentioned fluids was used. The fluid was put in the vase (the filtration system). Muassel (10 g) was put in the water-pipe head, and covered by a piece of pierced aluminum foil. Each used foil had in total 35 pierced holes. Two coals (50 g) were burned and placed over the aluminum foil. A vacuum generator pressure was set at 300 pa and was used to generate suction. The generated smoke was allowed to pass through a small piece of cotton placed in the plastic tube (hose) for 30 minutes. After each run, the cotton piece was washed with methanol to extract the chemicals (toxins). The organic solvent was rotary evaporated under vacuum and the residue weight was recorded.

Determination of chemical constituents

TLC plates were used to predict the number of compounds in each extract. The mobile phase used was hexane: ethyl acetate (4:1). The spots were detected using a UV lamp at 254 nm. LC-MS analysis was performed on the filtration fluids (water, tea, and silica solution) and the number of compounds (peaks) was recorded. Moreover, the instrument was applied to identify the presence of certain chemical compounds including nicotine.

Cytotoxicity method

MTS assay

Cells were seeded in sterile 96- well plates at a seeding density of 1 × 10³ cells per well in double concentrated and incubated for 24 h. After that, each extract (DWE and SSE) was given at 300, 100, 50, and 10 µg/ml concentrations in triplicates. After a 72-h incubation period, the anti-proliferative effect of the extracts was assessed by Cell Tilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay according to the manufacturer’s instructions (Promega Corporation, Madison, WI). At the end of the treatment, 20 µL of MTS solution/100 µL of media was added to each well and incubated at 37 °C for 2 h. The number of viable cells in each well was determined using a UV–Vis spectrophotometer measurements at 490 nm. The cell number was calculated using a standard curve. Cells treated with DMSO (0.1%) were used as controls for percent inhibition and CC₅₀ calculations (Shi et al. 2009).

Alpha-fetoprotein and apoptosis method

Hep3B hepatocellular cells were used as the HCC cell-line. The cells were purchased from ATCC (ATCC-HB-8064). Hep3B was cultured in DMEM with 10% fetal bovine serum, and secreted alpha-fetoprotein (αFP) served as a marker of tumor activity. Usually, the level of αFPs elevate in association with a variety of malignancies, especially within liver cancer cell lines, and the level of this protein is found in high numbers in untreated Hep3P liver cancer cell line. These cell lines were then exposed to the different tested cytotoxic extracts, and reduced αFP levels are the indicators of the extract having cytotoxic effects. The distilled water extract (DWE; regular water-pipe system) and silica solution extract (SSE) were used.

Procedure for smoke condensation using various filtration systems

To mimic the standard type of smoking (nargileh) used by the general community, a lab water-pipe (nargileh) was assembled using air suction and tobacco ignited coal. Several experiments were carried out to test the removal of smoke toxincants (tar and naphthalene derivatives). The following parameters were investigated; (i) the type of ignited coal, (ii) the amount and type of tobacco (10 g, Muassel), (iii) the volume of fluids used (200 ml of DW, tea, milk, sirup, silica solution, and sodium bicarbonate solution). All tests were run for 30 minutes and were done in duplicate. All experimental conditions were the same for all of the tests.
incubated for 24 h with Hep3B at a concentration of 10 μl/ml, and then medium sFP levels were assessed by a commercially available ELISA kit from R&D Systems, Inc., USA. Hep3B was then harvested, trypsinized (0.05% trypsin/0.53 mM EDTA), washed, and analyzed for apoptosis (Liu et al. 2010). After culturing, the harvested Hep3B cells were adjusted to 10⁶/ml in staining buffer (in saline containing 1% bovine albumin; Biological Industries, Israel). To determine Hep3B purity, they were fixed with 4% paraformaldehyde for 10 minutes and permeabilized with 0.1% saponin in PBS for 20 minutes, then stained with anti-human HBsAg monoclonal-antibody (R&D systems, USA) for 30 min at room temperature. For apoptosis and viability measurements, propidium-iodide (PI) staining of fragmented DNA and phosphatidylserine staining by annexin V-conjugated to FITC (R&D Systems, Minneapolis, MN) were used according to the manufacturer’s instructions. Apoptosis was defined as annexin-V (+), but propidium-iodide (-). Viable cells were defined as annexin-V (-) but propidium-iodide (-). In each experimental setting, unstained controls and IgG isotype controls as well as FMO controls were used (Waters et al. 1998, Hawash et al. 2020).

Electrophysiology of AMPAR

Whole-Cell recordings

Methods including DNA preparation, cDNA transient transfection, and cell culturing of human embryonic kidney cells (HEK293) expressing the flip isoform of AMPAR subunits have been previously described. 36 to 48 hours after transfecting the HEK293 cells (replated on laminin-coated coverslips), electrophysiological recordings were documented. GFP fluorescing cells were chosen for current recordings carried out by a giga-seal using borosilicate glass to create patch electrodes with 2–3 MΩ resistance. Whole-cell patch-clamp recordings were obtained via an integrated patch amplifier IPA (Sutter Instruments, Novato, CA) and SutterPatch Software v. 1.1.1 to digitize membrane currents for a short period at 22°C, –60 mV membrane potential, 10 kHz sampling frequency, and a low pass filter set at 2 kHz. Extracellular solution consisted of 150 NaCl, 2.8 KCl, 0.5 MgCl₂, 2 CaCl₂, and 10 HEPES, adjusted to pH 7.4 with NaOH (all concentrations measured in mM), while pipette solution included 10 CsF, 30 CsCl, 4 NaCl, 0.5 CaCl₂, 10 trypsin – EDTA solution B (0.25%), EDTA (0.05%), and 10 HEPES, adjusted to pH 7.2 with CsOH (mM concentrations). A double-barrel glass (theta tube) and high-speed piezo solution switcher (Automate Scientific, Berkeley, CA) allowed for a constant wash out of the cell while supplying it with our desired compound. Solution exchange ranging from 10–90% occurring at 500 ms, represented by the open tip potential recorded during the application of solutions of different ionic strengths that resulted from the patch’s expulsion from the electrode to estimate the solution’s speed exchange. The sample size included 6 viable cells used to record the mean inhibition by the derivative of interest. Cell health and data validation were ensured by resupplying the cell with the control (glutamate) after each agonist washout, producing results similar to glutamate-induced currents readings carried out before exposing the cells to the studied compounds (data in the supplemental material). Data acquired were analyzed using Igor Pro7 (Wave Metrics, inc). Receptor desensitization (tdes) and deactivation rates were estimated by a single exponential fitting of the current decay, starting from 95% of the peak to the baseline current. The currents were evoked by applying 10 mM glutamate for desensitization (500 ms) and 20 ms of the same concentrated glutamate for deactivation (Qneibi et al. 2019, 2020).

Results

Chemical results

Water pipe smoking, generally used water to filter out some of the incoming toxins from the tobacco smoke. However, other filtration fluids can be used to absorb the smoke’s toxicant. To our knowledge, there are no previous mentions of different filtration media in water-pipe smoking other than water, hence, we aim to replace water as a filtration media with other fluid types to reduce the amount of chemicals inhaled. The efficacy of the filtration system was evaluated by calculating the number of compounds in the collected residues, identifying their composition, and quantifying their amount.

Determining the extract quantity

The amounts of extracts in milligrams were measured for each collected fluids. The organic extractions were dried, and the amount of the residue for each filtration system was recorded (see Figure 2). Results showed that the amount of the extracted residue (which will reach the smoker’s lung) of different filtration systems was variable indicating the filtration capacity of the fluid system used. The silica solution (SSE) showed the smallest accumulation of chemicals extracted (90 and 57.5 mg) using 10 g and 50 g solution systems respectively. On the other hand, the standard water system (DWS) showed 154.5 mg of chemical deposit. The ‘no solution’ and milk solution filtration systems showed the highest chemical concentration accumulations of 445.5 and 436.1 mg, respectively (Figure 2). The TLC and LC-MS results of the tested filtration systems are provided in the supplemental file.
The most important filtration systems were the normal filtration system (distilled water extract: DWE; control), tea filtration system (Tea solution extract: TSE) and developed filtration system (silica solution extract: SSE), and the main chemical results of these two systems were listed in Table 1. However, chemical results obtained from the LC-MS demonstrated that an unknown compound with molecular weight 353.16 g/mol was not present in SSE (fewer peaks were observed in the LC-MS reading) in comparison with DWE and TSE systems.

Cytotoxicity results

MTS assay

To study the cytotoxic impact and activity of the SSE in relation to the standard DWE system, both filtration techniques were tested on three cell lines: HEK293t Normal embryonic kidney, MCF-7 breast cancer, and Hep3B liver cancer cell lines. Four concentrations of extracts were used to calculate the CC50 for these extracts, and it was clear that the DWE was more toxic against all cell lines HEK293t, MCF3B and MCF7 with CC50 values 77.1, 3.1, and 5.24 μg/ml, in comparison to SSE CC50 values 149.9, 10.14 and 8.9 μg/ml, respectively (Table 2). To further associate the inhibitory effects of DWE and SSE on Hep3B cell proliferation, medium levels of αFP were evaluated. Both DWE and SSE reduced Hep3B secretions of αFP to 1658.6 ± 141 ng/ml and 2273 ± 121 ng/ml, respectively, as compared to 4066 ± 202 ng/ml in untreated cells. This indicates less cell growth and reduced tumorigenicity of Hep3B cell line following treatment (Table 2), yet our results show that the SSE is less cytotoxic in comparison with the DWE (p < 0.08).

To determine whether DWE and SSE induced apoptosis (programmed cell death) and necrosis, annexin-V test was carried out. Cells undergoing apoptosis have their phosphati-dylserine (PS) phospholipid translocated from the inner face of the plasma membrane to the cell surface. Therefore, apoptotic cells can be identified by the presence of PS on the cell surface. As mentioned in the materials and methods section, detection of PS was estimated by staining with a fluorescent conjugate of annexin-V, a protein that has a high affinity for PS, followed by flow cytometry analysis. Cells were also stained with propidium iodide (PI), which can enter the cell only when the plasma membrane is damaged. Early apoptosis was evaluated as positive for PS but negative for PI. This was distinguished from late apoptotic and necrotic cells that were estimated as positive for both PS and PI. Figure 3 illustrates apoptosis (annexin-V+/PI-) fraction from DWE and SSE significantly increased to 55.67 ± 6%, and 49.33 ± 2.1%, respectively, as compared to 49.0 ± 2% in untreated cells (Hep3B cancer cell line). The reducing of necrosis populations of annexin-V-/PI- from 31.33 ± 3.78% in the untreated cells to 15.5 ± 3.5% in the DWE and 13.67 ± 3.78% in the SSE treatments (Figure 3) (p < 0.05). Our data suggest that both extracts have cytotoxic effects on Hep3B cells, specifically, DWE increases cell death in comparison with SSE, which means DWE has more potent anti-proliferative activities (more toxic) on Hep3B.

Biophysical gating results

Level of inhibition from DWE vs SSE on AMPA-Type receptors

DWE and SSE extractions were tested on AMPARs infected HEK293 cells. The cells are first washed with 10 μM of glutamate alone, inducing the opening or activation of ~95% of AMPARs (Qneibi et al. 2012), and resulting in the current reading. We then compare the current recordings with amplitudes generated by washing the cells with 50 μM of glutamate and DWE, or 50 μM of glutamate and SSE, and run a one-way ANOVA test for inhibition data significance. Our result shows that cells containing GluA2 homomer-type receptors experience a 4-fold amplitude drop when exposed to DWE, undergoing an amplitude drop from 1250 ± 72 pA to 255 ± 32 pA (Figure 4(A,B)). GluA1/2 heteromeric receptors also undergo amplitude inhibition, dropping from 530 ± 29 pA to 161 ± 19 pA, hence only reducing the amplitude by a 3-fold drop. Amplitude drops with SSE exposure are noted to be less significant for both GluA2 and GluA1/2 receptor-types. A 2-fold current drop is observed with GluA2 AMPAR (1312 ± 67 pA to 624 ± 41 pA), while an even less impressive 1-fold drop in GluA1/2 receptors from 560 ± 42 pA to 466 ± 25 pA (with SSE). Generally, it appears as GluA2 receptors are more greatly impacted by exposure to the smoke toxins.

Effect of DWE and SSE on AMPAR desensitization and deactivation

To further understand the impacts of DWE and SSE toxins on GluA2 and GluA1/2 AMPARs, we studied the exposure of said toxins on the receptors’ biophysical gating properties. AMPAR-current desensitization and deactivation were fitted with two exponentials, and the weighted tau (τw) was calculated as τw = (τf × af) + (τs × as), where af and as are the relative amplitudes of the fast (τf) and slow (τs) exponential components. In GluA2 type receptors, both DWE and SSE toxins impact desensitization and deactivation time (Figure 4(C,D)). However, DWE toxins are more successful in significantly increasing time spent in these inactive states, as desensitization rates increased from 2.7 ± 0.1 ms to 4.8 ± 0.4 ms with DWE, and 3.9 ± 0.4 ms with SSE. Deactivation rates increased from 2.3 ± 0.1 ms to 4.1 ± 0.3 ms and 3.2 ± 0.2 ms with DWE and SSE, respectively. With GluA1/2, only DWE washes significantly impacted AMPARs gating mechanisms. Desensitization rates increased from 4.8 ± 0.2 ms to 6.2 ± 0.4 ms and 4.8 ± 0.3 ms, while deactivation rates increased 2.6 ± 0.1 ms to 3.3 ± 0.2 ms and 2.7 ± 0.2 ms for DWE and SSE, respectively. Our findings indicate that GluA2 type receptors, relative to GluA1/2 receptors, are more greatly impacted by DWE toxins than SSE toxins.

Discussion

There is no clear evidence that confirms the significant role of water as a filtration system in a water-pipe (Kadhum et al. 2015). However, our observation and results demonstrate that water does reduce inhaled chemicals from heated...
tobacco, but still unproductive. The results showed a difference in quantities of collected extract using normal water-pipe (DWE) compared to the ‘no solution’ water-pipe findings (154 mg and 445 mg, respectively). In the DWE system, water-soluble chemicals that remained in the water (filter medium) were hence not inhaled. This explains the darkening of the color of water in the water-pipe vase with time. In contrast, with the ‘no solution’ water-pipe, all chemicals and toxins were collected in the cotton piece (445 mg). This was the highest quantity of chemicals collected among all different solutions. Since results showed water has a remarkable reduction of toxic material compared to no solution it was worth examining the filtration capacity of different solutions to improve the filtration of the inhaled smoke.

In the commonly used filtered system (DWE), results show that the 154.3 ± 2 mg of toxins collected in the cotton piece are expected to reach lung tissues. Therefore, the development of health disorders associated is expected with long-term exposure to water-pipe smoke if this filtration system is maintained. Therefore, using other filtration media is essential to reduce these hazards. Various filtration systems were tested compared to the standard system, with the most significant results obtained from the silica solution system (SSE). An evident decline in chemicals was witnessed when SSE was used in comparison with DWE, as SSE reduces the number of toxicants from 154 to 57.65 mg, and according to the LC-MS spectrum, one unknown chemical was disappeared in SSE compared with DWE. Naphthalene derivatives (tar; M.Wt. 174.16 g/mol), and nicotine (M.Wt. 163.12 g/mol) were very clear in the LC-MS results in all tested extracts (DWE, SSE, and TSE), while the unknown compound (M.Wt. 353.15 g/mol) disappeared in the SSE in comparison with DWE and TSE. Therefore, we hypothesized a difference in SSE impact on biological activity relative to the standard Water-pipe system (DWE).

Various harmful effects of smoking have been identified, including increased risk of cardiovascular disease development (El-Zayadi 2006). To determine the cytotoxic effect of the smoking extract, the different filtration techniques were tested on cell lines, with the main cell lines used being the Hep3B cancer cell line. Cytotoxicity results show that condensed smoke from the water-pipe on various cell lines is harmful and can decrease cell viability in various cell lines (HEK293T, MCF-7 and Hep3B). DWE has significant CC50 values 77.0, 3.1, and 1.2 μg/ml on the mentioned cells respectively, and the condensed smoke generated decreases α-FP from 4066.67 (the value of untreated cells) to 1658.67 ng/ml. However, the cytotoxic results of the developed filtration system (SSE) significantly reduce the impact on the cell lines (CC50 149.9, 10.1, and 8.8 μg/ml on the mentioned cells) when compared to DWE values, and this condensed smoke decreases α-FP from 4066.67 (the value of untreated cells) to 2273.33 ng/ml. More so, while apoptosis is considered a naturally occurring physiological process and necrosis a pathological one, usually caused by external agents like toxins, trauma, and infections (Jaeschke and Lemasters 2003, Malhi et al. 2006), DWE toxins increase the necrotic effect by 15.5% in comparison to SSE toxins (13.7% increase) on the cancer cell line (Hep3B). Hence, we can assume that water-pipe condensed smoke DWE has a more harmful impact on cells than the developed system SSE.

Another significant effect of the toxic impacts of water-pipe smoke is observed on neuronal AMPARs. Our results show that DWE toxins were remarkably effective in inhibiting and reducing the activity of GluA2 and GluA1/2 type receptors in HEK293 cells, specifically in GluA2 receptor variants. Given that GluA2-receptor types are the most prevalent type

toxicology, but still unproductive. The results showed a difference in quantities of collected extract using normal water-pipe (DWE) compared to the ‘no solution’ water-pipe findings (154 mg and 445 mg, respectively). In the DWE system, water-soluble chemicals that remained in the water (filter medium) were hence not inhaled. This explains the darkening of the color of water in the water-pipe vase with time. In contrast, with the ‘no solution’ water-pipe, all chemicals and toxins were collected in the cotton piece (445 mg). This was the highest quantity of chemicals collected among all different solutions. Since results showed water has a remarkable reduction of toxic material compared to no solution it was worth examining the filtration capacity of different solutions to improve the filtration of the inhaled smoke.

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| Cell line | SSE | DWE | Control |
|-----------|-----|-----|---------|
| HEK293T   | 149.913 ± 3.49 | 77.081 ± 3.28 | 2.32 ± 1.3 |
| MCF-7     | 10.143 ± 1.04  | 3.131 ± 0.91  | 0.314 ± 0.51 |
| Hep3B     | 8.878 ± 2.79   | 5.248 ± 0.76   | 0.314 ± 1.0  |
| α-FP (ng/ml) | 2273.33 ± 121.025 | 1658.67 ± 141.50 | 4066.67 ± 202.07 |

Table 2. The CC50 of the two filtration systems (DWE and SSE) against three cell lines, and alphafeto protein level in comparison with positive control (dox and untreated cells).

Table 1. The chemical results of the most significant filtration systems.
receptors in the brain, our findings suggest that these receptors will undergo significant inactivation, preventing necessary inter and intraneuronal cell signaling and communication (Zhao et al. 2019). This disruption of AMPAR signaling, or neuronal homeostasis, may contribute to the development of neurodegenerative diseases such as Alzheimer’s disease, epilepsy, and neuronal ischemic death (Tau and Peterson 2010, Qneibi et al. 2019). Further concerns lay in the fact that a significant number of smoking participants are in their youth (Al-Rawi et al. 2018). Most biological components, including the nervous system, undergo major development and modifications during this period, hence deactivating GluA2 and GluA1/2 receptor activity could only be viewed as detrimental to the development of the nervous system circuitry and specific processes such as memory, both means that are heavily reliant on AMPARs (Zhao et al. 2019). Interestingly, SSE contents showed no significant impacts on GluA1/2 receptors, and reduced effects on GluA2 receptors, indicating that its use as a filtration method in the water pipe might ameliorate the negative impacts of smoking.

Conclusions
Our study shows that condensed smoke produced by the water pipe has significant harmful effects on different cell lines. We can conclude that filtration media (such as water) in the shisha/water pipe reduces the number of toxic compounds that reach smokers’ lungs. Our experimental results demonstrate that using different filtration media can reduce toxins’ concentration inhaled by smokers. Substituting the traditional water-pipes (DWE) with silica in water (SSE) greatly reduces the quantity and number of toxins that are inhaled by smokers, and adding silica beads to the water medium increases the ability of the solution to dissolve more toxins and therefore increases the filtration. More so, the cytotoxicity results confirm the theory that water can filter the toxic compounds, yet the silica solution system is more effective in absorbing greater quantities of toxic compounds, which leads to less cell toxicity. Additionally, AMPARs GluA2 and GluA1/2 receptor variants were negatively impacted by DWE and SSE toxins. GluA2-type receptors were more significantly impacted in general and showed more significant inhibition when exposed to DWE contents rather than SSE contents, further supporting the importance of implementing the silica filtration system that may protect neuronal cells and reduce or prevent neurodegenerative disease development. Finally, the use of a better filtration system such as SSE may be effective in reducing the harmful effects of smoking a water pipe, given the ability of the SSE system to dissolve the unknown compound (353.16 g/mol) in comparison with standard system DWE. In the future, we aim to separate this unknown compound from the extracts, characterize its chemical characteristics, and evaluate its biological impacts.

Figure 4. DWS and SSE’s inhibitory effect on different AMPA-type subunits and their effect on desensitization and deactivation. (A) show the whole-cell recording of amplitude after applying the cell with 10 mM glutamate (Glu) alone (black), with glutamate plus 50 μM of DWE (light gray), and glutamate plus 50 μM of SSE (dark gray). (B) Inhibition assays of DWE and SSE’s on GluA2 and GluA1/2. (C) DWS and SSE affect AMPARs desensitization weighted time constants (τw des) at 500 ms. (D) Effect DWS and SSE on AMPA receptors deactivation weighted time constants (τw deact) at 1 ms. Desensitization and deactivation time constants changes are observed in different AMPAR-type subunits when exposed to glutamate (Glu) alone (10 mM) or Glu + DWE and SSE solutions (50 μM). The whole-cell current was recorded in whole-cell patched HEK293-expressing homomeric GluA2 and Heteromeric GluA1/2 alone or in combination with DWS and SSE solutions. It was conducted at -60 mV, pH 7.4, and 22 °C. Graphs summarize weighted time constants for activation. Data shown are mean ± SEM; n = 6–8 (number of patch cells in the whole-cell configuration). Significance (one-way ANOVA): *p < 0.05; **p < 0.01; ***p < 0.001; ns, not significant.
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No potential conflict of interest was reported by the author(s).

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Available upon request.

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