A comparative study of wood sawdust and plastic smoke particulate matter with a focus on spectroscopic, fluorescent, oxidative, and neuroactive properties

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Abstract

Here, water-suspended smoke aerosol preparation was synthesized from biomass-based fuel, i.e., a widespread product for residential heating, wood sawdust (WP) (pine, poplar, and birch mixture), and its properties were compared in parallel experiments with the smoke preparation from plastics (PP). Molecular groups in the PM preparations were analyzed using Raman and Fourier-transform infrared spectroscopy. WP was assessed in neurotoxicity studies using rat cortex nerve terminals (synaptosomes). Generation of spontaneous and H₂O₂-evoked reactive oxygen species (ROS) detected using fluorescent dye 2',7'-dichlorofluorescein in nerve terminals was decreased by WP. In comparison with PP, WP demonstrated more pronounced reduction of spontaneous and H₂O₂-evoked ROS production. WP completely inhibited glutamate receptor agonist kainate-induced ROS production, thereby affecting the glutamate receptor-mediated signaling pathways. WP decreased the synaptosomal membrane potential in fluorimetric experiments and the synaptosomal transporter-mediated uptake of excitatory and inhibitory neurotransmitters, L-[¹⁴C]glutamate and [³H] γ-aminobutyric acid (GABA), respectively. PP decreased the ambient synaptosomal level of [³H]GABA, whereas it did not change that of L-[¹⁴C]glutamate. Principal difference between WP and PP was found in their ability to influence the ambient synaptosomal level of [³H]GABA (an increase and decrease, respectively), thereby showing riskiness in mitigation of synaptic inhibition by PP and triggering development of neuropathology.

Keywords Air pollution particulate matter · Wood sawdust · Plastics · Oxidative potential · Neurotoxicity · Environmentally derived health threats

Abbreviations

PM	Particulate matter
WP	Water-suspended wood sawdust smoke aerosol preparations
PP	Water-suspended plastic smoke aerosol preparations
GABA	γ-Aminobutyric acid
EDTA	Ethylenediaminetetraacetic acid

HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
ROS	Reactive oxygen species
DCF	2',7'-Dichlorofluorescein
UV-Vis	Ultraviolet-visible

Introduction

Neurological disorders are the 3rd reason of disability and premature death in the European Union, and this burden is expected to be increased (Deuschl et al. 2020; Karthika et al. 2021) and significantly aggravated by COVID pandemic because of “long COVID” neurological complications (Jarrahi et al. 2020; The Lancet Neurology 2021). Etiology of neurological disorders still remains unclear, and their stable expansion is linked to air pollution with PM_{2.5} (particulate matter, size of which is less than

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2.5 μm), which disperses globally, traveling across state boundaries, oceans, and continents (Jiang et al. 2015; Zhang et al. 2017), targeting nervous system, and triggering development of neurological disorders and complications, e.g., lowered cognitive function, autism, neurodegenerative disease, dementia, and stroke (Landrigan et al. 2018). A possibility of interaction of SARS-CoV-2 with air pollution PM serving as a potential carrier for transmission of SARS-CoV-2 immobilized at their surface to the nervous system, and neurological symptom enhancer is also not excluded (Borisova and Komisarenko 2020; Rahman et al., 2020).

One of the main sources of carbon-containing PM emissions is biomass burning aerosol that mainly includes fires of forests, agricultural waste, and wood combustion for residential heating spread in developed countries (Fuzzi et al. 2015). Wood combustion and biomass burning contribution to organic carbon at European sites is between 30 and 75% (Gilardoni et al. 2016), and water-suspended carbon of air pollution can also pollute water resources worldwide. Chemical and physical features of wood burning particles vary in dependence on the combustion conditions and biomass types. Wood is utilized for fuel as conventional wood (woodchips) and wood pellets made from compacted sawdust (Saosee et al. 2020). Wood pellets are gaining attractiveness as a biomass-based fuel for heat and electricity production (Thraen et al. 2017; Sun and Niquidet 2017) and the wood pellet demand has enlarged resulting in its increased production (Jonsson and Rinaldi 2017). Global production of wood pellets increased considerably by 14% per year since 2011 (Thraen et al. 2017) and was estimated as 52.7 million tonnes in 2018 (“World Pellet Map – European Pellet Council,” 2021).

A large part of “synthetic” precursors of carbon-containing PM is plastics that is still utilized being subjected to open-fire combustion. Very recently, we synthesized in the laboratory conditions water-suspended smoke samples from plastic, which were appropriate for biological experiments and contained major nano-sized (30 nm) PM fraction. The smoke samples from plastic revealed oxidative activity in brain nerve terminals and decreased the membrane potential and synaptic vesicle acidification and functioning of excitatory/inhibitory neurotransmitter transporters, thereby provoking presynaptic malfunctioning and triggering neurological consequences (Borysov et al. 2020). Glutamate and γ -aminobutyric acid (GABA) are the crucial neurotransmitters for excitatory and inhibitory signal transduction in the central nervous system. Impairments of their transport parameters are involved in pathogenesis of main neurological complications and disorders. Engineered carbon-containing nanoparticles synthesized by combustion of organics also significantly dysregulated the abovementioned presynaptic processes (Borisova et al. 2015, 2017; Borisova 2019).

The aims of this study were as follows: (1) to collect in the laboratory conditions water-suspended smoke aerosol preparations from wood sawdust (pine, poplar, and birch mixture) (WP) using recently developed method (Borysov et al. 2020) for water-suspended plastic smoke PM preparations (PP); (2) to define the average size of PM in WP using dynamic light scattering; (3) to analyze optical and fluorescence properties, and Raman and Fourier-transform infrared (FTIR) spectra of WP, (4) to assess neurotoxic properties studying its activity in isolated rat cortex nerve terminals (synaptosomes), in particular, to analyze oxidative properties and key characteristics that determine synaptic neurotransmission, namely, Na^+ -dependent transporter-mediated uptake and the ambient levels of glutamate and GABA; and (4) to compare above WP characteristics with PP ones in parallel experiments.

Methods and materials

Laboratory synthesis and collection of wood sawdust smoke aerosol

Smoke of wood sawdust (pine, poplar, and birch mixture) was collected in the laboratory conditions during the entire flaming, mixed combustion phase (when the flaming and smoldering phases are present at the same time), and smoldering phase. Smoke emissions were monitored using Air Quality Monitor/Mini Laser $\text{PM}_{2.5}$ Monitor/SDL607. Wood (10 g) was combusted, smoke emissions were collected, and 4000 ml of smoke aerosol was sampled and bubbled through 5 ml of water. WP enriched with nano-sized PM were obtained by filtration through Millipore filters (0.45 μm) (Borysov et al. 2020), because the most harmful to human health is the nano-sized PM (Borisova 2018). The obtained WP was dried to measure the concentration of particulate smoke components.

Dynamic light scattering

Particle size in WP was examined using dynamic light scattering with a laser correlation spectrometer Zetasizer-3, Malvern Instruments (UK) equipped with He–Ne laser LGN-111 ($p = 25 \text{ mW}$, $k = 633 \text{ nm}$) according to (Borysov et al. 2020).

Optical and fluorescent properties

The optical properties of WP and PP were obtained with ultraviolet–visible absorption and photoluminescence spectroscopy using a Lambda Bio (PerkinElmer) spectrophotometer and QuantaMaster (PTI) spectrofluorimeter.

Raman and FTIR spectroscopy

The Raman spectra of WP were recorded using a custom-made Raman instrument pre-verified and equipped with a 40× objective microscope. A Verdi G laser, Coherent Inc. with a wavelength of 532 nm was used to excite the WP; the laser power on the sample was 30 mW. The scattered light from the sample was collected by the lens and filtered using two filters (RazorEdge 0° Longpass filter, Semrock). Raman scattered light was focused on a monochromator inlet slit (IsoPlane 320, Princeton Instruments) set at 30 μm to deflect unfocused light and have a high spectral resolution. The monochromator was equipped with a diffraction grating of 600 lines/mm, and the calculated spectral resolution was approximately 2 cm⁻¹. A CCD (PyLoN: 400BR-eXcelon CCD, Princeton Instruments), cryogenically cooled at -120 °C, was applied as detector.

WP was analyzed using FTIR spectrometer INVENIO-R (Bruker, Germany). Samples for the Raman scattering spectra and infrared absorption were prepared by applying a drop of WP solution to a CaF₂ substrate.

Ethical statement in animal experiments

Males, Wistar rats, with body weight of 100–120 g, were kept in a temperature-controlled room at 22–23 °C using the institutional facilities, and they were provided ad libitum with water and dry food. All experiments with animals were carried out according to the ARRIVE guidelines (McGrath et al. 2010). The protocol was approved by the Animal Care and Use Committee of the Institute, the Protocol #5 from 01/09/2020. Total number of rats used in this study was 30. To save animals, synaptosomal preparations were shared between radiolabeled and fluorescence experiments. As a result, 9 separate rats were used for analysis of the L-[¹⁴C] glutamate uptake; 3 separate rats and 6 shared rats — [³H] GABA uptake; 12 separate rats — the ambient levels of L-[¹⁴C]glutamate and [³H]GABA; 6 separate rats and 3 shared rats — fluorescence measurements.

Inclusion and exclusion criteria

There were no exclusions in each experimental group of animals. The exact value of *n* in each experimental group was indicated in the figures and tables. The quality of synaptosomal isolation procedure from rat brain and synaptosome viability was controlled in each experiment by specific criteria, i.e., the value of the ambient level of L-[¹⁴C]glutamate in the control nerve terminal preparations that characterized active dynamic glutamate turnover across the plasma membrane of nerve terminals (Borisova and Borysov 2016; Borisova 2016). There were no data points excluded from analyses because of their biologically implausible values.

The rat cortex nerve terminals (synaptosomes)

The synaptosomal preparations were obtained by differential and Ficoll-400 density gradient centrifugation of homogenates according to the method described by Cotman (Cotman 1974) with slight modifications (Borisova and Himmelreich 2005; Borisova 2014; Tarasenko et al. 2010). The protein concentrations were monitored according to Larson (Larson et al. 1986). WP aliquots were added to the synaptosomal suspensions and incubated for 10 min.

The synaptosomal membrane potential (E_m)

The membrane potential of synaptosomes was measured using the potentiometric fluorescent dye rhodamine 6G (0.5 μM) based on its potential-dependent binding to the membranes (Borysov et al. 2014, 2018; Pozdnyakova et al. 2016). Fluorescence measurements of rhodamine 6G were carried out using a fluorescence spectrofluorometer QuantaMaster™ 40 (PTI, Inc., Canada) at 528 nm (excitation) and 551 nm (emission) wavelengths.

Reactive oxygen species (ROS) in nerve terminals

A cell-permeable non-fluorescent probe, 2',7'-dichlorodihydro-fluoresceindiacetate (H2-DCFDA), was applied to measure ROS generation in synaptosomes according to (Borysov et al. 2020). 2',7'-dichlorofluorescein (DCF) fluorescence alterations were recorded at excitation and emission wavelengths of 502 and 525 nm, respectively (slit bands were 2 nm each) using a fluorescence spectrofluorometer QuantaMaster™ 40 (PTI, Inc., Canada).

The transporter-mediated L-[¹⁴C]glutamate uptake by nerve terminals

The uptake was initiated by the addition of 10 μM glutamate supplemented with L-[¹⁴C]glutamate (420 nM, 0.1 μCi/ml). The synaptosomal transporter-mediated L-[¹⁴C]glutamate uptake was measured using liquid scintillation counting with Sigma-Fluor® High Performance LSC Cocktail (Soldatkin et al. 2015).

The ambient level of L-[¹⁴C]glutamate in nerve terminal preparations

The synaptosomes were loaded with L-[¹⁴C]glutamate (1 nmol/mg of protein, 238 mCi/mmol) in the standard saline solution at 37 °C for 10 min. The ambient levels of L-[¹⁴C]glutamate in the synaptosomal preparations were measured according to (Borisova et al. 2016; Borisova 2018) using liquid scintillation counting with Sigma-Fluor® High Performance LSC Cocktail.

The transporter-mediated [^3H]GABA uptake by nerve terminals

The uptake was initiated by the addition of GABA and [^3H]GABA (1 μM or 50 nM and 4.7 $\mu\text{Ci}/\text{ml}$, respectively) and terminated by filtering aliquots through Whatman GF/C filters as described in (Pozdnyakova 2017).

The ambient level of [^3H]GABA in nerve terminal preparations

The synaptosomes were loaded with [^3H]GABA (50 nM, 4.7 $\mu\text{Ci}/\text{ml}$) in the standard saline solution for 10 min. [^3H]GABA radioactivity was measured in aliquots of supernatants by liquid scintillation counting with Sigma-Fluor® High Performance LSC Cocktail according to (Pozdnyakova et al. 2015).

Statistical analysis

The results are expressed as the mean $\pm$ S.E.M. of n independent experiments. Experimental data on the effects of control vs WP or PP were analyzed using one-way ANOVA. The accepted level of significance was set at $p < 0.05$.

Materials

EGTA, EDTA, HEPES, Ficoll 400, aminooxiacetic acid, D-glucose, sucrose, Whatman GF/C filters, the fluorescent dye 2',7'-dichlorofluorescein, Sigma-Fluor® High Performance LSC Cocktail, organic counting scintillant (OCS)

and the analytical grade salts were purchased from Sigma (St. Louis, MO, USA); L- $^{14}\text{C}(\text{U})$ glutamate, [^3H]GABA (γ -[2,3- $^3\text{H}(\text{N})$]-aminobutyric acid) were from PerkinElmer (Waltham, MA, USA). Rhodamine 6G were obtained from Molecular Probes (USA).

Results

Size of particles in WP

Analysis of the average size of particles in WP was performed by dynamic light scattering. Distribution of particles' population by number (Fig. 1) was assessed and peak analysis demonstrated that the major number of particles in WP was 29.7 nm in diameter. Studying PP, the similar distribution of particles' population by number was revealed, where the major number of particles was 34 nm in diameter (Borysov et al. 2020).

Optical and fluorescent properties of WP and PP

The optical properties of WP were characterized using ultraviolet–visible (UV–Vis) absorption and photoluminescence spectroscopy. Figure 2a represents UV–Vis absorption spectrum of WP (versus PP) recorded with a Lambda Bio (Perkin Elmer) spectrophotometer. Similarly to PP, the absorbance of WP was mainly registered in the UV region (generally within the range of 200–350 nm), but in contrast to PP, it had a pronounced peak at around of 275 nm. Photoluminescence experiments carried out using QuantaMaster (PTI) spectrofluorimeter have demonstrated that

Fig. 1 Dynamic light scattering histograms of WP: distribution of particle populations by number. Five measurements each during 1 min, sequentially 1, red; 2, blue; 3, green; 4, grey; 5, purple; are present in each histogram. The measurements were performed with a Zetasizer Nanosystem (Malvern Instruments) equipped with a helium–neon laser

Fig. 2 **a** UV–Vis absorption spectra of WP and PP; **b** Photoluminescence (PL) emission spectrum of WP (pine wood) at excitation of 275 nm. **c** PL emission spectra of WP and **d** β -alanine carbon dots. Insets show emission spectra normalized to their maximum value (a

curve fitting was done using ‘GCAS’ function, OriginPro.9.0), and **d** also demonstrates the absorption spectrum of β -alanine carbon dots (black curve)

the excitation of WP at 275 nm led to fluorescence emission with a maximum wavelength at about 340 nm that is typical for phenolic derivatives (Fig. 2b). The strong absorbance in the UV region 200–400 nm, followed by a tail extended to the visible region, is known to be a typical feature of carbon dots (Liu et al. 2020), which may be produced during the thermal decomposition of wood. Indeed, the production of several types of carbogenic materials including carbon dots, as a result of hydrothermal carbonization of cellulose, was earlier reported (Woo et al. 2020). Given that one of the unique properties of carbon dots are the dependence of the photoluminescence emission maximum on the excitation wavelength (λ_{exc}), we next

recorded emission spectra of WP at λ_{exc} progressively increased from 300 to 400 nm. Figure 2c clearly shows that an increase in λ_{exc} led to a red-shift of the photoluminescence emission maximum from approximately 430 to 500 nm, thereby demonstrating the excitation-wavelength-dependent photoluminescence behavior typical for carbon dots. These data were similar to those we reported earlier for PP (Borysov et al. 2020), as well as to data obtained for carbon nanoparticles derived from thermal decomposition of β -alanine (Fig. 2d). As Fig. 2d depicts, β -alanine carbon nanoparticles absorbed light in a narrow UV-region and similarly with WP, it showed the strong excitation wavelength dependent emission properties.

Raman and FTIR spectroscopy of WP and PP

WP experiments

Using Raman spectroscopy, bands of 1353 cm^{-1} (D-band) and 1597 cm^{-1} (G-band) were registered during analysis of separate components of WP (Fig. 3a). These bands were a characteristic for carbon nanodots formed during combustion and however depended on the degree of wood burning, and also, other products of oxidation and uncompleted combustion appeared in the spectra on the background of luminescence (Fig. 3b, c).

FTIR spectra were corrected for baseline and normalized to the C=O band in the region of 1715 cm^{-1} (Fig. 4). In

general, Raman and FTIR spectra revealed similar chemical groups in WP (Figs. 3 and 4, Tables 1 and 2).

PP experiments

Table 2 demonstrates frequencies of PP. Raman and FTIR spectra of PP were presented in our previous study in the Supplementary Materials (Borysov et al. 2020).

The bands seen in the smoke suspension spectra were mainly related to lignin and its pyrolysis products. Analysis of the FTIR absorption spectra of separate components of WP was performed in two areas $3800\text{--}2400\text{ cm}^{-1}$ and $1850\text{--}950\text{ cm}^{-1}$. The absorption region with a maximum in the range of $3406\text{--}3383\text{ cm}^{-1}$ belonged to the molecular

Fig. 3 Raman spectra of separate WP components, i.e., birch (a), poplar (b) and pine (c) wood

Fig. 4 FTIR spectra of separate WP components, i.e., birch (green line), poplar (blue line), and pine (red line) wood, obtained in the range of 3800–2300 cm^{-1} (a) and 1850–950 cm^{-1} (b)

groups of OH, which is part of the phenolic, hydroxyl, and carboxyl groups (Zhong and Jang 2014; Fan et al. 2016). In addition, bands of stretching vibrations of aliphatic CH_2 (2907 and 2843 cm^{-1}) and CH_3 (2966, 2945, and 2880 cm^{-1}) molecular groups have been registered in this region. In particular, the band 2869 cm^{-1} can be attributed to the methoxy group ($\text{O}-\text{CH}_3$) of lignin (Dias Júnior et al. 2019). The contribution of CH_2 groups of poplar wood was slightly greater than that of birch and pine woods. The

band in the region 1715–1717 cm^{-1} referred to the valence vibrations of the molecular group $\text{C}=\text{O}$, which was a part of the carboxyl groups ($\text{HO}-\text{C}=\text{O}$) and carbonyl groups ($\text{H}-\text{C}=\text{O}$) of aldehydes and ketones (Zhong and Jang 2014; Fan et al. 2016). In untreated wood, this band made a much smaller contribution and was in the higher frequency position (1720–1725 cm^{-1}) and referred to unbound (unconjugated) $\text{C}=\text{O}$. After the pyrolysis process, there was an increase in the contribution of $\text{C}=\text{O}$ and its low-frequency

Table 1 Frequencies of separate WP components

Birch	Poplar	Pine	Frequencies
3406			str OH
	3383	3380	str OH
2962	2966	2965	str CH ₃
2942	2945	2944	str CH ₃
2912	2907	2911	str CH ₂
2880	2880	2883	str CH ₃
2869	2869	2869	str O-CH ₃ lignin
2843	2843	2843	str CH ₂
1728			str C=O carboxyl
1715	1717	1715	str C=O carboxyl
		1702	str C=O ketonic and/or aldehydic
1682	1678	1682	str C=O
1551	1658	1652	str C=O, C=C ketones, lignin
1613	1608	1613	str C=C
	1593	1600	str C=C
1516	1516	1516	str C=C lignin
1463	1460	1455	asym def CH ₃ in lignin
1429	1429	1433	def CH in lignin and carbohydrates, def CH ₂
1368	1372	1363	def CH in cellulose and hemicellulose, carbohydrates,
1331	1331	1331	C-O
1277	1273	1274	C-O
	1240	1238	C-O lignin
1217	1220	1212	Str C-O-C syringyl ring
	1165		C-O-C cellulose and hemicellulose
	1136	1135	C-O-C lignin
1114	1115		Aromatic skeletal and str C-O lignin and polysaccharides
		1081	C-O
1052	1052	1049	C-O cellulose and hemicellulose, polysaccharides
997			
933			

shift, which was associated with the degradation of lateral lignin ligaments (Kubovský et al. 2020). The bands in the region of 1680–1650 cm⁻¹ and the intense band in the region of 1613 cm⁻¹ indicated the presence of unsaturated bonds in aromatic rings, including lignin and polysaccharide degradation products. At increased temperature, this 1613 band was mainly unchanged (Dias Júnior et al. 2019). The band in the region of 1500 cm⁻¹ referred to the aromatic C=C valence vibrations of benzene rings of lignin that in the process of pyrolysis shifted to a higher frequency region (in this study, the band of 1516 cm⁻¹). This band was also associated with the syringyl nucleus of lignin, which was a characteristic of harder woods. Bands in the area of 1516 cm⁻¹ and 1114 cm⁻¹ (C-O lignin) were considered markers of the presence in the atmosphere of smoke from the combustion of biomass, in particular wood (Fan et al. 2016). The region 1463–1368 cm⁻¹ can be attributed to the deformation oscillations of different groups of CH. In particular, the band in the region 1463–1455 referred

to the deformation of aromatic CH and asymmetric lignin CH₃ groups. The band in the region 1429–1433 cm⁻¹ can be attributed to deformation of CH₂ groups in cellulose and deformation of CH groups in lignin and carbohydrates. The band in the region 1372–1363 cm⁻¹ was also a deformation CH groups in carbohydrates, in cellulose and hemicellulose (Timar et al. 2016); 1331 cm⁻¹ C-O valence vibrations in the syringyl parts of lignin; 1240–1238 cm⁻¹ C-O valence vibrations of lignin; 1165 cm⁻¹ — C-O-C valence vibrations in cellulose and hemicellulose; 1135–1136 cm⁻¹ — C-O-C valence vibrations of lignin (Boeriu et al. 2004; Derkacheva and Sukhov 2008). The spectra of birch and poplar preparations were very similar, in contrast to the spectrum of pine preparation. In particular, this applied to the band 1272 cm⁻¹, which was more intense than the band 1217 cm⁻¹ in the spectrum of pine preparation, as compared to birch and poplar preparations, and also, a redistribution of component deposits in the range from 1150 to 1000 cm⁻¹.

Table 2 Frequencies of PP

PP	Frequencies
3375	str OH
3283	str
3186	str
3061	str C-H aromatic
2983	str CH ₃
2955	str CH ₃
2916	str CH ₂
2848	str CH ₂
2642	
2530	
	str C=O
1731	str -C=O
1707	str -C=O
1686	str -C=O
1648	str C=O
	str C=C
1596	str C=C
1546	str C=C
1504	str C=C
1450	asym def CH ₃
1408	def CH, def CH ₂
1316	
1295	
1268	C-O
1177	C-O-C
1134	
1095	C-O
1071	
1018	
943	
927	

Burning plastics releases the products of decomposition and oxidation of the polymers. The higher the combustion temperature, the higher the variety of types of combustion products is. In the PET sample before combustion, the characteristic bands were the deformation vibration of the CH in the range of 1370–1340 cm⁻¹, the contribution of which can determine the presence of amorphous and crystalline phases in the plastic. Plastic smokes were characterized by the presence of wide absorption band in the region of 3400 cm⁻¹, which referred to the stretching vibrations of hydrogen-bound OH molecular groups. The region of 3000–3100 cm⁻¹, referred to CH ring vibrations and the region of 2920–2880 cm⁻¹, to the stretching vibrations of CH₂, CH₃. The region of 1800–1650 cm⁻¹ was the absorption of C=O molecular groups. According to literature data in the original plastic, the C=O vibration was located at 1720 cm⁻¹, and after combustion, it was at 1685 cm⁻¹, and there was a very intense shoulder in 1731 cm⁻¹ in plastic

smoke (Fabia et al. 2020), and these literature data are in accordance with our spectra (Fig. 4, Table 2).

Neuroactive properties of WP

Spontaneous and induced ROS generation in nerve terminals in the presence of WP

To elucidate whether WP influenced redox state of nerve terminals and to compare obtained results with action of PP, the kinetics of ROS generation was monitored using fluorescent dye H₂-DCFDA (see “Methods and materials” section). Figure 5a compares the effects of WP (poplar wood) versus PP on the level of ROS, which are spontaneously generated by nerve terminals due to their normal metabolic activity. WP (like PP) significantly and dose-dependently decreased spontaneous ROS production; however, it showed much higher antioxidant efficiency than PP.

Antioxidant effect of WP became much more pronounced after the application of a highly reactive hydrogen peroxide to synaptosomes. As shown in Fig. 5b, pre-incubation of synaptosomes with different aliquots of WP significantly (up to 100%) inhibited ROS generation induced by H₂O₂ (50 μM). Similarly with spontaneous ROS production, WP also decreased H₂O₂ action approximately 50 times more effectively than PP. It should be emphasized that such a powerful antioxidant effect of WP was observed regardless of whether it was preliminary added to the synaptosomes, or it was pre-incubated with H₂O₂ for 5–10 min before addition (data not shown).

It is important to note that WP was able to neutralize effectively ROS not only exogenously added to synaptosomes (like H₂O₂) but also generated within synaptosomes due to intracellular signaling pathways. Figure 5c depicts a dose-dependent effect of WP on the intracellular ROS generation in response to the addition of kainate (0.2 mM), an agonist of kainate/AMPA type glutamate receptors. As seen, pre-incubation of synaptosomes with high concentrations of WP completely inhibited kainate-induced ROS production, thereby affecting the receptor-mediated signaling pathways.

Effect of WP on the membrane potential of nerve terminals and H₂O₂-induced depolarization

The antioxidant properties of WP were also manifested when studying its effect on H₂O₂-induced depolarization of the synaptosomal membrane (Fig. 6). As shown in Fig. 6a, application of WP to the synaptosomes, pre-equilibrated with a membrane potential-sensitive fluorescent dye rhodamine 6G at a concentration of 20 μg/ml, did not significantly affect the membrane potential

Fig. 5 Comparison of the effects of WP (poplar wood) versus PP on the spontaneous (a) and 50 μM H_2O_2 -induced (b) ROS generation in nerve terminals. Effect of WP (pine wood) on kainate-induced ROS

generation (c). Each trace is representative of nine experimental data records performed with different synaptosomal preparations

but considerably reduced the depolarizing effect of H_2O_2 (100 μM) added 5min after WP. However, at higher concentrations, WP itself caused depolarization of the synaptosomal membrane and also inhibited the action of H_2O_2 much more significantly. As shown in the inset of Fig. 6a, the effect of WP was concentration-dependent and H_2O_2 -induced depolarization of the synaptosomal membrane was almost completely blocked by WP at a concentration of 100 $\mu\text{g}/\text{ml}$. As shown in Fig. 6b, WP was approximately 40 times more effective than PP regarding inhibitory action on H_2O_2 -induced depolarization of nerve terminal membrane, when comparing WP versus PP concentrations that caused similar effects. These data

confirmed that WP has a much higher antioxidant potential than PP.

Transporter-mediated uptake in the presence of WP: the initial rate and accumulation of L-[^{14}C]glutamate and [^3H]GABA by nerve terminals

Figure 7a shows WP-induced dose-dependent decrease in the initial rate of synaptosomal L-[^{14}C]glutamate uptake that was equal to 2.63 ± 0.18 nmol/min/mg of protein in control; 2.03 ± 0.17 nmol/min/mg of protein after application of WP at a concentration of 100 $\mu\text{g}/\text{ml}$ [$F_{(1,16)} = 5.88$; $p < 0.05$; $n = 9$]; and 1.78 ± 0.23 nmol/min/mg of protein

Fig. 6 **a** Dose-dependent effect of WP on the membrane potential of nerve terminals and 100 μM H₂O₂-induced membrane depolarization. Inset depicts H₂O₂-induced membrane depolarization at different WP concentrations after subtraction of their corresponding controls (application of water instead of H₂O₂). **b** Comparison of the effective

concentrations of WP versus PP, which caused a similar inhibition of H₂O₂-induced membrane depolarization of nerve terminals. Each trace is representative of nine experimental data records performed with different synaptosomal preparations

Fig. 7 The initial rate of synaptosomal transporter-mediated uptake and accumulation of L-[¹⁴C]glutamate (**a**) and [³H]GABA (**b**) after application of WP at different concentrations. Data is mean ± SEM. **p* < 0.05; ***p* < 0.01; ****p* < 0.001 as compared to control, *n* = 9

after application of WP at a concentration of 500 μg/ml [$F_{(1,16)} = 8.72$; $p < 0.01$]. Synaptosomal L-[¹⁴C]glutamate accumulation for 10 min was equal to 10.21 ± 0.52 nmol/mg of protein in control; 8.13 ± 0.39 nmol/mg of protein after application of WP (100 μg/ml) [$F_{(1,16)} = 11.64$; $p < 0.01$]; and 6.57 ± 0.63 nmol/mg of protein after application of WP (500 μg/ml) [$F_{(1,16)} = 22.09$; $p < 0.001$]. Therefore, the initial rate of synaptosomal uptake of L-[¹⁴C]glutamate and its accumulation decreased by 23% and 20%, respectively, after application of WP at a concentration of 100 μg/ml, and these parameters reduced by 32% and 35%, respectively at a WP concentration of 500 μg/ml.

The initial rate of synaptosomal [³H]GABA uptake was equal to 165.47 ± 10.95 pmol/min/mg of protein in control; 125.95 ± 8.58 pmol/min/mg of protein after application of WP (100 μg/ml) [$F_{(1,16)} = 6.7$; $p < 0.05$; $n = 9$]; 103.48 ± 13.47 pmol/min/mg of protein after application of WP (500 μg/ml) [$F_{(1,16)} = 10.43$; $p < 0.01$] (Fig. 7b). Synaptosomal [³H]GABA accumulation for 5 min was equal to 543.83 ± 29.67 pmol/mg of protein in control; 416.94 ± 32.17 pmol/mg of protein after application of WP (100 μg/ml) [$F_{(1,16)} = 6.42$; $p < 0.05$]; and 340.98 ± 13.75 pmol/mg of protein after application of WP (500 μg/ml) [$F_{(1,16)} = 43.29$; $p < 0.001$]. Therefore, both the initial rate of synaptosomal uptake of [³H]GABA and its accumulation decreased by 23% after application of WP at a concentration of 100 μg/ml, and both parameters reduced by 37% at a WP concentration of 500 μg/ml. In summary, WP components mitigated

functioning of both excitatory and inhibitory neurotransmitter transporters in nerve terminals.

Comparing with WP, PP also decreased uptake of both L-[^{14}C]glutamate and [^3H]GABA by nerve terminals in a similar way and range (Borysov et al. 2020).

A comparative study of the ambient levels of L-[^{14}C]glutamate and [^3H]GABA in nerve terminal preparations in the presence of WP and PP

L-[^{14}C]glutamate assay

It was revealed that the ambient levels of L-[^{14}C]glutamate in synaptosomal preparations in the presence of WP and PP were not changed considerably and were equal to $16.93 \pm 0.66\%$ of total accumulated L-[^{14}C]glutamate in control; $16.74 \pm 0.68\%$ [$F_{(1,22)} = 0.04$; $p = 0.85$; $n = 12$], and $18.91 \pm 0.66\%$ [$F_{(1,22)} = 2.14$; $p = 0.16$; $n = 12$] after application of 100 and 500 $\mu\text{g/ml}$ WP, respectively. The ambient levels of L-[^{14}C]glutamate were equal to $17.29 \pm 0.84\%$ [$F_{(1,22)} = 0.11$; $p = 0.74$; $n = 12$] of total accumulated label after application of 100 $\mu\text{g/ml}$ PP, and $17.86 \pm 1.01\%$ [$F_{(1,22)} = 0.59$; $p = 0.45$; $n = 12$] after application of 500 $\mu\text{g/ml}$ PP (Fig. 8a).

[^3H]GABA assay

The ambient levels of [^3H]GABA in synaptosomal preparations after application of WP were changed and consisted of $10.78 \pm 0.35\%$ of total accumulated [^3H]GABA in control; $14.36 \pm 1.08\%$ [$F_{(1,22)} = 10.88$; $p < 0.01$; $n = 12$] after application of 100 $\mu\text{g/ml}$ WP; and $29.64 \pm 1.65\%$ [$F_{(1,22)} = 138.3$; $p < 0.001$; $n = 12$] after application of 500 $\mu\text{g/ml}$ WP (Fig. 8b).

Whereas, oppositely directed changes as compared to WP were shown in the ambient levels of [^3H]GABA in synaptosomal preparations after application of PP, and it was revealed that PP decreased the ambient [^3H]GABA level that consisted of $10.11 \pm 0.47\%$ of total accumulated [^3H]GABA

[$F_{(1,22)} = 1.43$; $p = 0.24$; $n = 12$] after application of 100 $\mu\text{g/ml}$ PP and $7.45 \pm 0.34\%$ [$F_{(1,22)} = 50.27$; $p < 0.001$; $n = 12$] after application of 500 $\mu\text{g/ml}$ PP (Fig. 8b).

Discussion

In this study, a comparative assessment of the spectroscopic, fluorescent, oxidative, and neuroactive properties of WP and PP was carried out. Data on dynamic light scattering revealed that WP similarly with PP consisted of major fraction of nano-sized particles (Fig. 1). In this context, both WP and PP possessed unique characteristics inherent to nano-sized particles that in turn can influence their environmental distribution and health effects. Literature data have confirmed that ultrafine air pollution $\text{PM}_{0.1}$ (size of which is less than $0.1 \mu\text{m}$) is extra hazardous to human health in comparison with fine $\text{PM}_{2.5}$ and coarse PM_{10} (Fine et al. 2004; Borisova 2018). The smaller the size of airborne PM, the stronger toxicity through mechanisms of oxidative stress and inflammation can be registered (Valavandis et al. 2008). Both WP and PP demonstrated the main optical absorption in the UV region within the range of 200–350 nm, but in contrast to PP, WP had a pronounced peak at around 275 nm (Fig. 2). Such high-energy absorbance band may be attributed to guaiacol- and syringol-type compounds, which are the major components of wood smoke and are formed as a result of lignin degradation. Lignin is a branched biopolymer that together with hemicelluloses and pectin acts as an adhesive matrix for cellulose microfibrils. In softwood, lignin consists mainly of guaiacil units, i.e., the structure of lignin is derived from coniferyl alcohol, while hardwood shells consist mainly of syringil derived from mustard alcohol. The main components of wood were cellulose (45–60%), hemicellulose (15–35%) and lignin (in conifers up to 35% by weight in deciduous 20–25% respectively) (Hon and Shiraishi 2001; Nedukha 2015; Janusz et al. 2017). Absorption data from Fig. 2 were confirmed by Raman and FT6R spectroscopy (Figs. 3 and 4, Tables 1 and 2).

Fig. 8 The ambient levels of L-[^{14}C]glutamate (a) and [^3H]GABA (b) in synaptosomal suspensions in the control group and after application of WP and PP (100–500 $\mu\text{g/ml}$). The data are the mean $\pm$ SEM. n.s., no significant differences; $**p < 0.01$; $***p < 0.001$ as compared to control; $n = 12$

Oxidative properties of WP and PP in nerve terminals were unidirectional but had different efficiency levels (Fig. 5a, b). Comparative analysis of the antioxidant activities of WP versus PP revealed that WP showed much higher antioxidant efficiency than PP in relation to both the spontaneous and induced ROS generation. This may be at least partially explained by the presence in WP not only carbon dots, which were reported to possess antioxidant properties, but also a large amount of methoxyphenols, the structural features of which were similar to those of phenolic antioxidants, such as ubiquinol and tocopherols (Kjällstrand and Petersson 2001). Our findings are consistent with literature data, where plastic organics and carbon nanodots demonstrated antioxidant properties by scavenging oxidant free radicals and ROS, and the carboxyl and amino groups were involved through hydrogen atom transfer reaction (Li et al. 2018; Zhang et al. 2018; Ji et al. 2019). As our data demonstrate, WP potently inhibited an increase in ROS generation induced by the activation of kainate-type glutamate receptors, which are known to modulate synaptic transmission and synaptogenesis (Fig. 5c). In particular, we have previously shown that presynaptic glutamate receptor-mediated ROS production was tightly coupled with the modulation of GABA release from cortical and hippocampal nerve terminals (Tarasenko et al. 2012). In this context, and given the numerous evidence about the important role of ROS in the regulation of neurotransmission and synaptic plasticity (Massaad and Klann 2011; Beckhauser et al. 2016; González et al. 2020), the inhibition of kainate-induced ROS production by WP may lead to the disruption of receptor-mediated signaling pathways and so indirectly affects neuronal synaptic activity.

One of the main findings of this study is the fact that PP were able to decrease the ambient level of [^3H]GABA (Fig. 8a), whereas the ambient level of L-[^{14}C]glutamate remained unchanged (Fig. 8b), and WP did not change both parameters (Fig. 8a, b). It should be emphasized that the extracellular level of neurotransmitters between the episodes of exocytotic release is a balance between transporter-mediated uptake/release and the tonic leakage of neurotransmitters and is a very important characteristic that determines strengths of synaptic contacts and regulates synaptic neurotransmission (Borisova and Borysov 2016; Borisova 2016). Neurochemical mechanisms of the PP-induced attenuation of the ambient [^3H]GABA level in nerve terminals (Fig. 8b) despite decreased [^3H]GABA uptake (Fig. 7b) can be suggested but needs further detailed examination. Our previous study has revealed a tight correlation between ROS production and GABA secretion in nerve terminals (Tarasenko et al. 2012). A possible explanation was that PP components, but not WP ones, can decrease tonic [^3H]GABA leakage from nerve terminals, thereby compensating a decrease in [^3H]GABA uptake. Therefore, WP and PP demonstrated

neurotoxic features and can provoke malfunction at the presynapse via weakening transporter-mediated glutamate/GABA uptake by nerve terminals that misbalanced excitatory and inhibitory neurotransmission. Moreover, PP can provoke a decrease in synaptic inhibition via lowering ambient level of GABA in the synaptic cleft. These facts can relate to recently shown association of air pollution by PM with nervous system disorders and neurodegenerative disease, including dementia, cognitive function reduction, attention deficit and hyperactivity in children, autism, stroke, nausea, and headaches (Verma et al. 2016; Landrigan et al. 2018). The central nervous system is not secured against evolutionary new challenge of intensive exposure to anthropogenic carbon-containing air pollution PM (Borisova 2018). Particles can be deposited in the nasal region of humans and move along olfactory nerve axons straight to the brain circumventing the blood brain barrier (Oberdörster et al. 2005). Also, our data is in accordance with our previous 18, where we have shown recently in modelling experiments that carbon nanoparticles synthesized by heating of carbohydrates (beta-alanine and thiourea) possessed neurotoxic effects (Borisova et al. 2015, 2017). In perspectives, our further efforts will be focused on the analysis of individual effects of separate components of wood sawdust smoke PM on synaptic transmission. We suggest that different types of wood in sawdust can have different effects on excitatory and inhibitory signaling and so differently influence synaptic neurotransmission.

Conclusion

WP were synthesized from a widespread biomass-based fuel for residential heating, wood sawdust. Synthesized WP contained the major nano-sized PM fraction. Light absorption and fluorescent properties of WP were characterized in details. Neurotoxicity studies using nerve terminals demonstrated that WP attenuated the membrane potential and demonstrated more pronounced reduction of spontaneous and H_2O_2 -evoked ROS production as compared to PP, thereby influencing synaptosomal oxidative processes. WP and PP provoked presynaptic malfunction reducing the uptake of glutamate and GABA that in turn can misbalance excitatory and inhibitory signaling. Moreover, PP can provoke a decrease in synaptic inhibition via lowering ambient level of GABA in the synaptic cleft. Therefore, WP and PP demonstrated similarity in the PM size, optical properties, effects on the membrane potential, L-[^{14}C]glutamate/[^3H]GABA uptake and the ambient L-[^{14}C]glutamate level, whereas principal difference was found in synaptosomal ambient level of [^3H]GABA between WP and PP that can be a reason for development of neuropathology in response to human exposure to air pollution PM (Table 3).

Table 3 Comparison of WP and PP properties

Property	WP	PP
Size of PM	30 nm	34 nm
Optical properties (absorbance)	One peak in the region 200–270 nm	One peak in the region 250–300 nm
Fluorescent properties	Strong photo luminescence in the visible and near-infrared range	Strong photoluminescence in the mid infrared range
The synaptosomal membrane potential	Decreased	Decreased
Spontaneous and H ₂ O ₂ -evoked ROS generation in nerve terminals	Decreased to the lesser extent than PP	Decreased
L-[¹⁴ C]glutamate/[³ H]GABA uptake by nerve terminals	Decreased	Decreased
The ambient level of L-[¹⁴ C]glutamate in nerve terminals	Not changed	Not changed
The ambient level of [³ H]GABA in nerve terminals	Not changed	Decreased

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Author contribution Synthesis and collection of WP and PP, measurements of their size, monitoring PM_{2.5} emission were carried out by AB and KP; dynamic light scattering, AB; optical properties of WP–KP and synaptosome isolation, AP; spectrofluorimetry using synaptosomes, AT; L-[¹⁴C]glutamate experiments, AP and NK; [³H]GABA experiments, NP; FTIR spectra, OS; Raman spectra, GD and OG; data analysis, TB, NP, NK, AT, OS, GD, and OG. Experimental design and draft paper writing was done by TB. The final version of the MS was revised and approved by all co-authors.

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Data Availability The authors declare that all data supporting the findings of this study are available within the article and its supplementary information files.

Declarations

Ethics approval All experiments using rats were conducted according to the Guidelines of the European Community (2010/63/EU), and the local laws and policies, and were preliminary approved by the Animal Care and Use Committee of the Institute (the Protocol #5 from 01/09/2020). The studies are in accordance with the ARRIVE guidelines for reporting experiments involving animals (McGrath et al. 2010).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare competing interests.

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