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Synthesis of calcium silicate hydrate from chicken eggshells and combined joint effect with nervous system insecticides

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ABSTRACT

The world will produce 90 million tons of chicken eggs in 2030. As eggshell represents 11%, about 10 million tons of waste shells accumulate in the environment, which causes a hazard to human health. Recycling this waste into value-added products requires adequate strategies. Herein, amorphous calcium silicate hydrate (ECSH) has been synthesized *via* sol-gel method by utilizing chicken eggshells as an alternative source of calcium. ECSH was characterized using the wide-angle X-ray diffraction (XRD) pattern, Fourier transform-infrared spectroscopy (FT-IR), N2 adsorption/desorption isotherms, and field-emission scanning electron microscopy (FE-SEM). The insecticidal activity of ECSH and nervous system insecticides (methomyl and chlorpyrifos) was evaluated against *Spodoptera littoralis.* Methomyl and chlorpyrifos displayed high acute toxic effects with LC_{25} of 158.0 and 98.14 ppm and LC_{50} of 438.25 and 256.68 ppm, respectively. The combination of ECSH with methomyl and chlorpyrifos increased the insecticidal activity and reduced the applied amount of such toxic insecticides.

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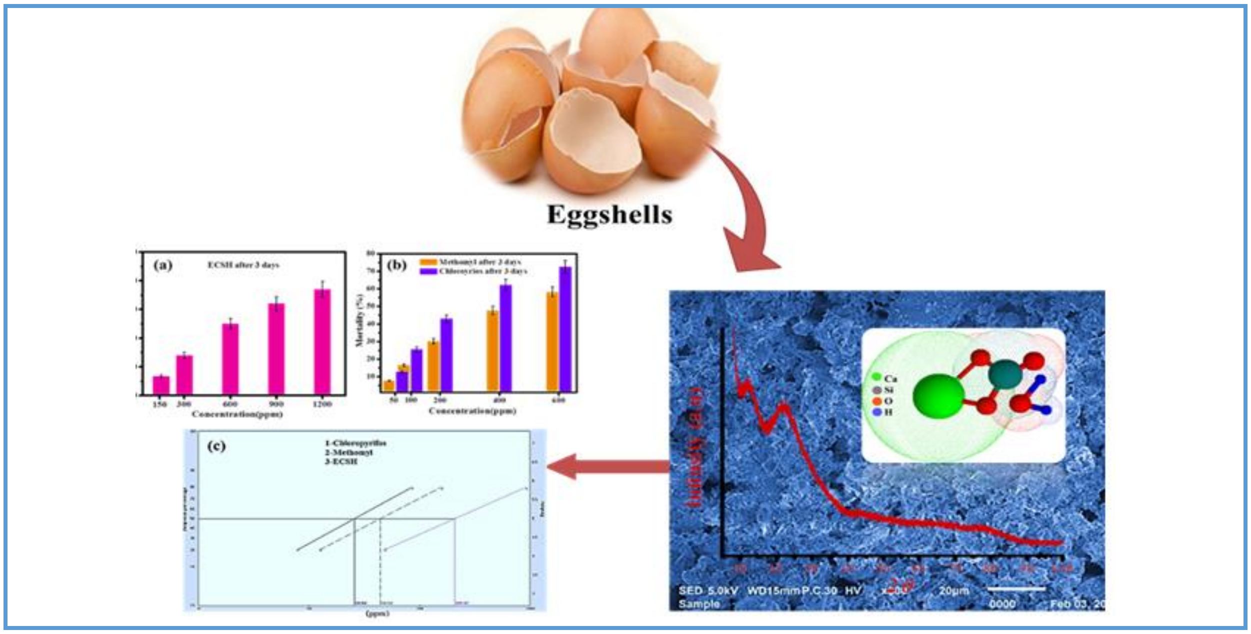
Graphical Abstract



Introduction

The current situation refers to the increased accumulation of chicken eggshell wastes without pre-treatment, causing a hazard to human health by the released odor from the environmental biodegradation [1, 2]. The global production of chicken eggs is expected to increase up to 90 million tons by 2030. Eggshell represents 11% (≈10 million tons of waste shells by 2030) of the total mass of the egg (5-6 g). It consists of calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%), and organic matter (4%), the recycling of this waste into value-added products requires adequate strategies to take into consideration [3–5]. Thus the reuse of eggshell waste as an alternative source of calcium produces both economic and environmental advantages [6]. Thermal treatment of waste eggshells to prepare calcium oxide opens a door for their application as a starting material for dielectrics such as $CaSiO_3$, $BaTiO_3$, $CaAl_2O_4$, and bio-catalysts [7]. As a biocompatible, bioactive, and biodegradable material, calcium silicates received significant attention in biomedical applications such as bone/dental restoration and drug delivery systems [8]. Calcium silicates such as wollastonite, olivine, and β-$CaSiO_3$, were considered bioactive materials due to their ability to form hydroxyapatite over their surface. In contrast, calcium silicate hydrate(CSH) is distinguished for the presence of OH groups and Ca^{2+} ions that react with the (PO_4^{3-}) groups providing high bioactivity [9, 10].

In another context, Pesticides are natural or synthetic agro-chemicals applied in different agricultural practices to control a wide range of pests (herbicides, insecticides, fungicides, rodenticides, and nematicides). Due to pest infestation, the world lost about 45% of its annual food production. Therefore, utilizing pesticides became an energetic agent for plant protection and improving crop yield [11, 12]. The world production of pesticides is increased by 11% per year. Three billion kilograms of pesticides are consumed every year; only 1% of total applied pesticides are effectively used to control the target pests. While the remaining amounts, 99%, run into the environment, causing negative impacts on human health [11, 13]. Anticholinesterase (AChE) is one of the nervous system insecticides widely applied in insect pest control programs. Carbamates and organophosphorus (OP) are two classes of anticholinesterases insecticides that exert neurotoxicity by inhibiting acetylcholinesterase enzyme [14]. Thus, acetylcholinesterase insecticides cause human death through the depression of respiratory centers in the brainstem and paralysis of the diaphragm if the inhibition of acetylcholinesterase enzyme is severe and sustained [15].

Therefore, in this work, we synthesized amorphous calcium silicate hydrate (ECSH) *via* sol-gel method by utilizing chicken eggshells as an alternative source of calcium. The insecticidal combined joint effect of ECSH with nervous system insecticides (methomyl and chlorpyrifos) was evaluated to increase their efficacy and reduce the applied amount.

Experimental

Synthesis of calcium silicate hydrate hydrate from chicken eggshells

Chicken eggshells were utilized as an alternative bio-calcium source to synthesize calcium silicate hydrate *via the* sol-gel method (Figure 1). The collected waste eggshells were washed with detergent and distilled water, dried at 80 °C overnight, and finally ground into a fine powder using porcelain mortar. An eggshell powder was thermally treated at a high temperature (800 °C) to prepare calcium oxide (CaO). A mixed solution of (10 mL TEOS, 40 mL EtOH, and 20 mL H_2O) was sonicated for 5 min to complete the hydrolysis. After that, A 30 mL of 0.1 M $CaCL_2$ was prepared by dissolving an appropriate amount of CaO in concentrated HCL and added to a silicate solution [16]. All the solutions were prepared using bi-distilled water and still under magnetic stirring for 4 h at 50 °C. The prepared calcium silicate hydrate was filtered, washed with water/ethanol three times, dried at 80 °C for 12 h, and finally calcined at 600 °C for 6 h to obtain amorphous calcium silicate hydrate (ECSH).

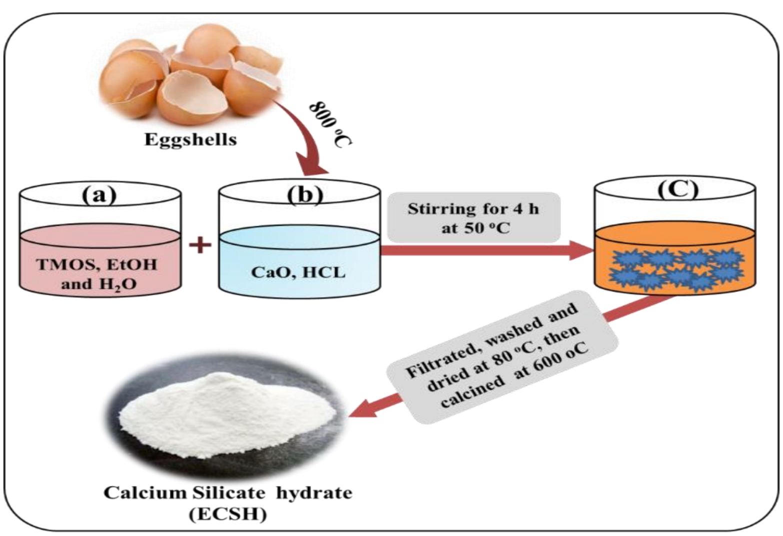


Figure 1. Schematic representation for the sol-gel synthesis of calcium silicate hydrate (ECSH) from waste eggshells

Characterization of calcium silicate hydrate

X-ray diffractometer (Model D8 Advance, Bruker) with monochromatic CuKα radiation (λ=1.54 Å), employing a scan rate of 0.06 min^{-1} was utilized to measure X-ray diffraction (XRD) patterns of calcium silicate hydrate.

Bruker Alpha Fourier transform-infrared (FT-IR) instrument was used to record calcium silicate hydrate FT-IR spectroscopy.

Calcium silicate hydrate surface properties were determined by N_2 adsorption/desorption isotherms at 73 K by using the BELSORP apparatus, Japan. Brunauer–Emmett–Teller (BET) method was utilized for calculating the specific surface area was calculated. At the same time, pore size distribution was determined from the analysis of the desorption branch of isotherm using the Barrett-Joyner-Halenda (BJH) method.

Field-emission scanning electron microscopy (FE-SEM, JEOL model 5400 LV) has been utilized for investigating the morphology of calcium silicate hydrate. High-resolution images were obtained via operating FE-SEM at 15 kV.

Insecticides

Nervous system insecticides (methomyl (HPLC, 99.7%) and chlorpyrifos (HPLC, 99.7%)) were received from Sigma-Aldrich Ltd., Germany.

Bioassay

The insecticidal efficacy of calcium silicate hydrate, methomyl, and chlorpyrifos was evaluated *via* the feeding bioassay method under the recommended conditions [17]. Series of different concentrations of each were prepared, and then leaf discs of a castor-bean plant were washed and dipped into the solutions. The leaves were introduced into a drying container containing equal numbers (20 larva/replica) of new molting 2nd instar larvae of *Spodoptera littoralis. T*he undipped leaves were marked as a control group.

Combined joint effect

Combinations are prepared by mixing LC_{25} or LC_{50} for methomyl and chlorpyrifos insecticide with calcium silicate hydrate *via* stirring for 30 minutes. The co-toxicity factors for the combined joint effect were calculated according to Equation (1).

$$\text{cotoxicity factor} = \frac{Observed\ mortality(\%) - Exepected\ mortality(\%)}{\text{Exepected mortality}(\%)} \times 100 \quad (1)$$

According to the co-toxicity factor, the joint effect was categorized as a potentiation effect if the co-toxicity factors >+20, antagonism effect if co-toxicity factors <-20, and additive effect if the factor in the range of ±20 [18].

Statistical analysis

Mortality percentages (three replicas/concentration) were calculated after three days of treatment, Abbott's formula was utilized to correct the natural mortality [19]. The sub-lethal (LC_{25}) and lethal (LC_{50}) concentrations were calculated by using the probit analysis program [20].

Results and Discussion

Characterization of calcium silicate hydrate

The wide-angle X-ray diffraction pattern (Figure 2**a**) shows that the as-prepared calcium silicate hydrate (ECSH) is amorphous [8]. Thus, the calcination at a relatively high temperature (600 °C) for ECSH produced an amorphous structure.

FT-IR spectra of ECSH showed characteristic absorption bands for the vibrational modes of the SiO_3 group that appear at 454 cm^{-1} for Si–O–Si and O–Si–O bonds, stretching modes of O–Si–O bonds at 795 cm^{-1} (Figure 2**b**). The symmetric stretching vibrations of Si–O–Si bonds and Si–O–Ca bonds were observed at 1115 cm^{-1}. The absorption band at 1411 cm^{-1} corresponding to the binding vibration of the C–O indicates the presence of ($-CO_3^{-2}$) groups. The band at 3434 cm^{-1} is attributed to the stretching vibration of Si–OH- - -H_2O; this band and the band at 1642 cm^{-1}indicate the presence of water in the chemical structure of calcium silicate [21, 22].

Textural properties, according to IUPAC classifications of adsorption isotherms, calcium silicate hydrate revealed type IV isotherm with a pronounced H_2 hysteresis loop which could characterize bottleneck constrictions (Figure 2**c**). The type of H_2 hysteresis is attributed to disordered materials, where the distribution of pore size and shape is not well defined. The specific surface area (S_{BET}) of ECSH was 362 m^2/g with a pore size of 2.21 nm (Figure 2**d**).

The surface morphology of calcium silicate hydrate was investigated by utilizing field-emission scanning electron microscopy (FE-SEM) (Figure 2**e** and **f**). Template-free sol-gel method produced highly dense aggregated sheets with different sizes of less than 5.0 µm of ECSH [16].

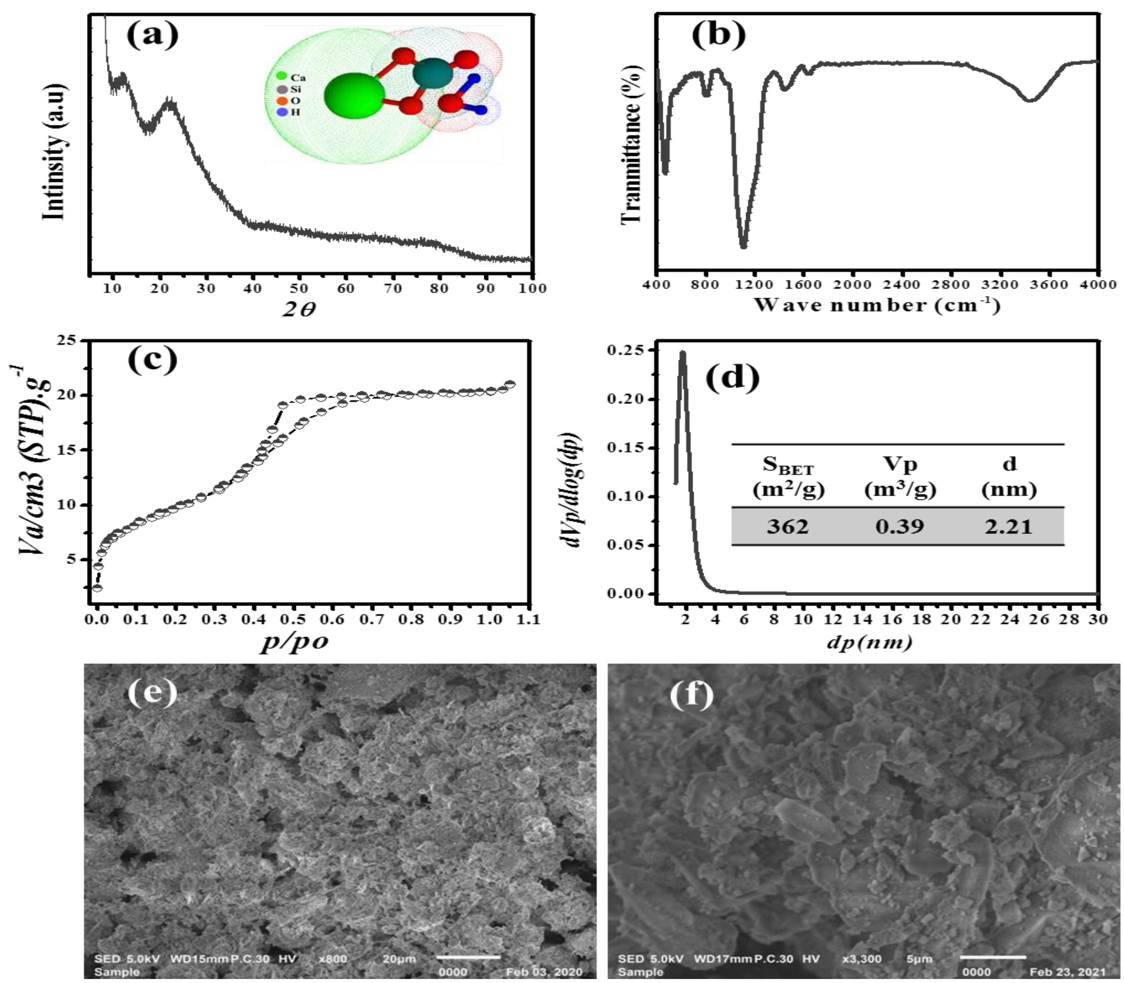


Figure 2. a) Wide-angle XRD, b) FTIR, c) N_2 adsorption/desorption isotherms, d) the corresponding pore size distribution curve, e,f) SEM images of ECSH

Insecticidal activity

Insecticidal activity of ECSH is mainly related to the impairment of the digestive tract and surface enlargement of the integument due to dehydration or blockage of spiracles and tracheas. In the aqueous suspensions, ECSH generates reactive oxygen radicals by cracking the particles in the silicon-oxygen bond. These radicals stabilized as a surface-bound reactive oxygen species and then decayed subsequently [23]. The mortality for 2nd instar larvae of *Spodoptera littoralis* showed a positive correlation with ECSH concentrations (Figure 3a). The sub-lethal (LC_{25}) and lethal (LC_{50}) concentration values were calculated as recommended for pesticide formulations using the probit analysis program (Table 1 and Figure 3c).

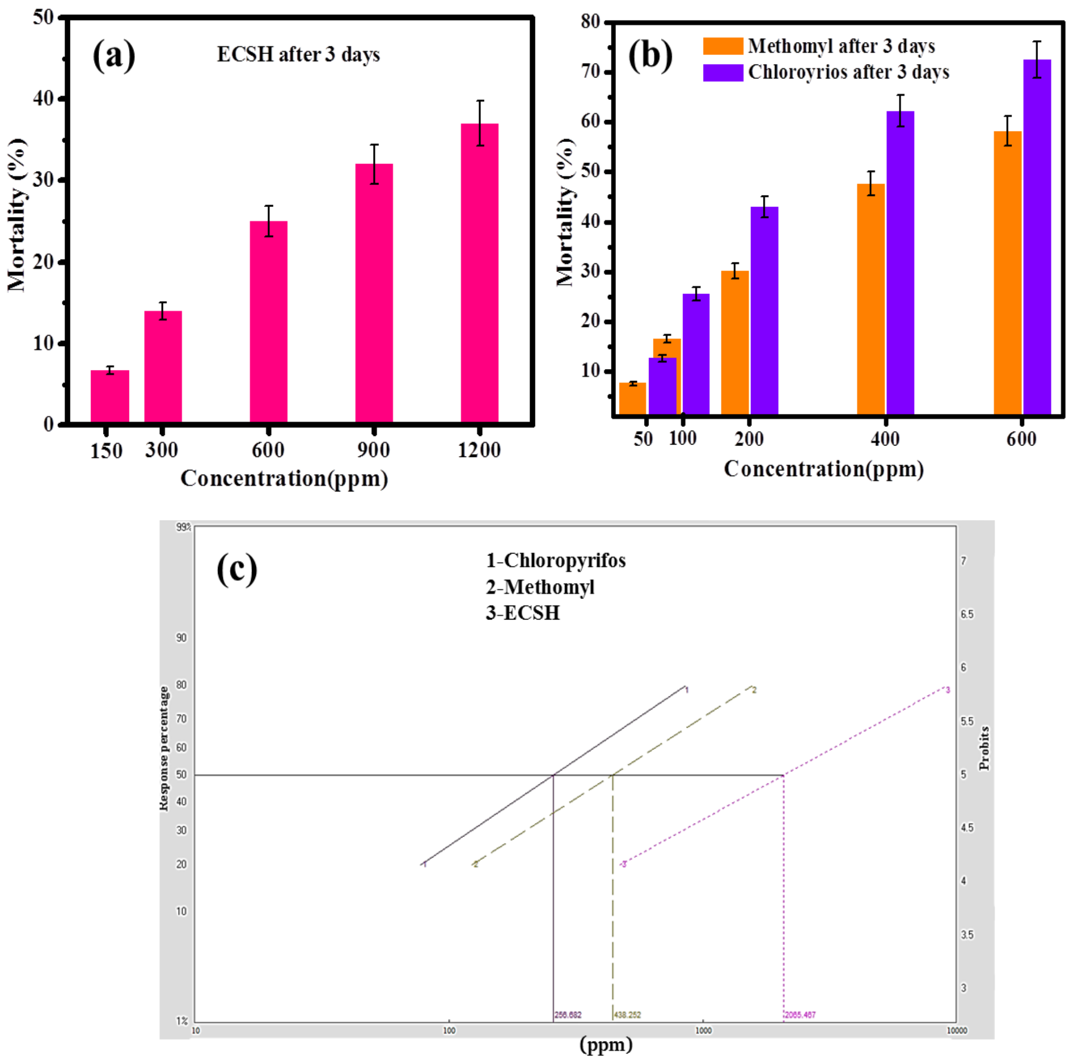


Figure 3. a) Mortality percentages of 2nd instar larvae of *S. littoralis* exposed to different concentrations of ECSH, b) methomyl and c) chlorpyrifos *via* leaf deep bioassay method after three days post-treatment, and the toxicity lines

Table 1. Toxicity data of 2nd instar larvae of *S. littoralis* exposed to different concentrations of ECSH, methomyl and chlorpyrifos

Insecticide	LC_{25} (ppm) (95% CL*)	LC_{50} (ppm) (95% CL*)	Slope ± SE	R	X^2	P
ECSH	630.04 (500.50-802.08)	2065.46 (1438.33-3989.27)	1.31± 0.22	0.999	0.027	0.99
Methomyl	158.00 (125.27-191.14)	438.25 (356.41-575.18)	1.52 ± 0.17	0.969	5.19	0.16
chlorpyrifos	98.14 (75.33 -120.11)	256.68 (216.73 - 309.12)	1.62 ± 0.16	0.969	7.63	0.05

*Confidence Limit

The insecticidal activity of methomyl and chlorpyrifos on 2nd instar larvae of *Spodoptera littoralis* was also evaluated three days post-treatment. The mortality percentages were concentration-dependent (Figure 3b). Methomyl and chlorpyrifos displayed high acute toxic effects with LC_{25} of 158.0 and 98.14 ppm and LC_{50} of 438.25 and 256.68 ppm, respectively (Table 1, and Figure 3c). The combination of ECSH with methomyl and chlorpyrifos increased their insecticidal activity compared to the individual insecticides (Table 2). Calcium silicate hydrate could rupture the cuticle layer, allowing methomyl and chlorpyrifos insecticides to penetrate the insect body. Thus, the miscellaneous mode of action resulted from the combination of ECSH with nervous system insecticides increases the insecticidal efficacy against *Spodoptera littoralis* which sequentially reduces the intensive usage of such highly toxic insecticides in crop protection programs.

Table 2. Joint effect of ECSH combined with methomyl and chlorpyrifos insecticides on *S. littoralis via* leaf deep bioassay method after three days post-treatment

Insecticide combination	Conc (ppm)	Observed mortality (%) ± SE	Co-toxicity factor	Joint effect
ECSH+Meth	LC_{25} + LC_{25}	68.06± 1.76	36.13	Potentiation
	LC_{50} + LC_{25}	76.03 ± 2.16	1.38	Additive
	LC_{25} + LC_{50}	81.56 ± 1.91	8.74	Additive
ECSH+Chlor	LC_{25} + LC_{25}	69.82 ± 2.04	39.64	Potentiation
	LC_{50} + LC_{25}	77.96 ± 1.86	3.94	Additive
	LC_{25} + LC_{50}	86.62 ± 2.28	15.48	Additive

Conclusions

Recycling chicken eggshell wastes into value-added materials produces both economic and environmental advantages *via* providing an alternative source of calcium and reducing the pollution caused by biodegradation. Amorphous calcium silicate hydrate (ECSH) was synthesized by utilizing the sol-gel method. ECSH was characterized using wide-angle X-ray diffraction pattern (XRD), Fourier transform-infrared(FT-IR), N2 adsorption/desorption isotherms, and field-emission scanning electron microscopy(FE-SEM). The insecticidal activity of ECSH was evaluated against *Spodoptera littoralis*. The insecticidal combined joint effect of ECSH with nervous system insecticides (methomyl and chlorpyrifos) was evaluated to increase the efficacy and reduce the applied amount.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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