Research Article

Transfer of Organochlorine Pesticide Residues during Household and Industrial Processing of Ginseng

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Ginseng is an important traditional herbal medicine; however, ginseng root may contain pesticide residues that may cause adverse health effects to consumers. Generally, people are more inclined to take the household- or industrial-processed ginseng products, instead of eating them directly. To investigate the intake of pesticides along with ginseng more specifically, we simulated two household processing methods (boiling and brewing) and two industrial processing methods (ethanol refluxing and boiling combined with resin purification) and then calculated the transfer rates of five organochlorine pesticide (OCP) residues in ginseng. The determination of targeted pesticide residues in ginseng was done by gas chromatography-electron capture detector (GC-ECD), and the confirmation was done by gas chromatography-tandem mass spectrometer (GC-MS/MS). The transfer rates of the OCPs in water extraction (boiling and brewing) were relatively low and would not increase significantly along with two hours of boiling. The OCPs were concentrated during the ethanol refluxing procedure because of the high transfer rates of the OCPs and the reduction of the weight of products. The boiling combined with resin purification method removed the OCPs most effectively. Different ginseng processing methods resulted in variable transfer rates of pesticides, as well as a diverse exposure risk of pesticides to humans. Consequently, it is necessary to concern about the transfer rates of pesticide residues during ginseng processing.

1. Introduction

Panax ginseng C. A. Mey. has been used as a nourishing and tonifying remedy for thousands of years in eastern Asia, including Korea, China, and Japan [1], and is currently distributed to 35 countries around the world [2]. In modern clinical medicine validation, ginseng is a nourishing drug that stimulates blood vessels, regulates nerves, promotes appetite, calms the brain, relieves fatigue, boosts metabolism, and enhances liver detoxification [3, 4]. With the enhancement of people’s self-care awareness and the improvement of their quality of life, ginseng is widely used not only in medicine and clinics but also in the cosmetics, food, beverage, and health care products [5–7]. According to recent research data from China Local Co., Ltd. [8], in 2018, the total ginseng production by China was 38,567 tons. In 2012, the China Health Ministry authenticated the cultivated ginseng as a new food resource with a recommended daily dose of 3 g [9], but the actual consumption may be more than that in some districts. Therefore, it is important for consumers to know the risk of intake of pesticides along with ginseng.

Black spot, sclerotinia, erythroderma, and root rot are major ginseng diseases, and organochlorine pesticides (OCPs), such as pentachloronitrobenzene, hexachlorocyclohexane, and hexachlorobenzene, have been used extensively to resist these diseases [10]. However, numerous experiments demonstrated that long-term exposure or consumption of substances containing OCPs can lead to many chronic diseases, such as nervous system disorders, cancer, reproductive toxicity, and birth defects [11, 12]. Although many countries have banned the use and sale of some OCPs, OCPs are highly resistant to...
degradation and may still remain in the environment [13]. Moreover, these persistent chemicals can be transferred and magnified to higher trophic levels through the food chain [14]. Organic matter in the soil can absorb and fix OCPs, which is the natural pool of OCPs in the environment [15]. Being planted in the soil containing OCPs, ginseng may absorb the organic chlorine pesticides in the soil and enrich them in various parts, which will affect the quality of ginseng products and ultimately endanger human health. Wang et al. [16] assessed the risk score of pesticide residues in ginseng according to the matrix ranking developed by the Veterinary Residues Committee of the United Kingdom, and they found that hexachlorobenzene, phorate, pentachloronitrobenzene, and BHC were classified as the high-risk group (risk score ≥28). Therefore, it is very necessary to study the organochlorine pesticides in ginseng.

It is reported that processing methods have different effects on pesticide residues in plants because of the physicochemical properties of pesticides, the characteristics of commodities, and the condition of processing [17, 18]. Common processing methods such as washing, peeling, blanching, and cooking contribute to the reduction of pesticide residues [19, 20]. Meanwhile, it is demonstrated that pesticide residues remaining in/on chili peppers are concentrated by the usual drying process [21]. Most researches of pesticide residues in ginseng focus on the crude ginseng material [16, 22], but people do not just simply consume raw ginseng. Boiling and brewing are usually employed in household ginseng processing, and ethanol refluxing and boiling combined with resin purification are commonly applied to industrial processing. Consequently, to assess the intake of pesticides along with ginseng more specifically, it is essential to investigate the transfer of pesticide residues during processing.

In this study, ginseng samples containing five OCPs (hexachlorobenzene (HCB), pentachloronitrobenzene (PCNB), α-BHC, γ-BHC, and δ-BHC) were used to simulate four processing methods as closely as possible. The pesticide residues in the raw ginseng, ginseng products, and dregs obtained by processing were determined by gas chromatography-electron capture detector (GC-ECD) and confirmed by gas chromatography-tandem mass spectrometer (GC-MS/MS). GC-ECD is highly sensitive to molecules like OCPs that contain electronegative atoms, and GC-MS/MS has advantages on identifying substances due to its specificity [23]. Then, factors affecting the transfer rates were analyzed, to understand the effects of OCPs’ physicochemical parameters, the characteristics of ginseng, and the condition of the processing method on OCPs’ transfer behavior. Results of our research have great importance in setting practical maximum residue limits (MRLs) and risk assessment in ginseng. Moreover, this case will provide a reference for other root commodities, such as ginger, yam, and salvia.

2. Materials and Methods

2.1. Instruments and Reagents. An ultrasonic bath (SB-2500DT, Ningbo Scientz Biotechnology Co., Ltd., China), centrifuge (TDZ5-WS, Hunan Xiang Yi Laboratory Instrument Development Co., Ltd., China), vortex mixer (VORTEX-5, Haimen Kylin-Bell Lab Instruments Co., Ltd., China), and rotary vacuum evaporator (LABOROTA 4000, Heidolph, Germany) were used. The gas chromatography-electron capture detector (GC-ECD) system was composed of an Agilent 7890A gas chromatograph equipped with a micro-electron-capture detector (μ-ECD) (Agilent, Inc., USA) and a DB1701 fused silica capillary column (30 m × 0.25 mm × 0.25 µm; Agilent, Inc., USA). The gas chromatography-tandem mass spectrometer (GC-MS/MS) system consisted of a Varian 450GC, a 300 triple-quadrupole MS (Bruker Daltonics, Inc., USA), and a capillary column DB-5 MS (30 m × 0.25 mm × 0.25 µm; Agilent, Inc., USA).

n-Hexane (HPLC grade) was obtained from Sigma Company (St. Louis, MO, USA); dichloromethane, acetone, petroleum ether, and anhydrous sodium sulfate (analytical grade) were obtained from Beijing Chemical Works Company (Beijing, China). The certified analytical standards of five OCPs were obtained from Agriculture Environmental Protection Institute, China. Structures and properties of five OCPs are shown in Figure 1. The percent purity of each pesticide is more than 99.0%. Separate liquid stock solution for each compound at the concentration of 100 mg·L⁻¹ in n-hexane was prepared and used for further dilutions. The stock and working solutions were stored hermetically at −20°C until analysis. The concentration of working mix pesticide standard solution is 0.02 mg·L⁻¹ in n-hexane.

2.2. Sampling. To investigate the transfer behavior of the OCPs scientifically, we need ginseng samples containing OCPs that were derived from field spraying during its process of growth. Therefore, batches of 6-year ginseng were collected from different local plantations in northeastern China, which is the main ginseng production area of China [24]. The concentrations of OCPs in these batches of ginseng were determined by GC-ECD (for quantitative analysis) and GC-MS/MS (for qualitative analysis). Then, we found one batch of ginseng containing a considerable amount of OCPs (hexachlorobenzene (HCB), pentachloronitrobenzene (PCNB), α-BHC, γ-BHC, and δ-BHC).

In the autumn of 2017, about 10 kg of fresh ginseng root was harvested from the local ginseng plantation where we found the batch of ginseng containing a considerable amount of OCPs; then, it was dried at 30−40°C. The dried ginseng root was cut into slight pieces, followed by mixing homogeneously, and raw ginseng was obtained. The raw ginseng was stored in plastic bags at −20°C for pesticide residue determination and performing four processing methods. The whole processing and pesticide residue determination procedure were finished within two months.

2.3. Four Processing Methods. There are three repetitions in each processing method to guarantee the accuracy of the research. The subsequent processing conditions were established as closely as possible to the actual conditions in practical production. The brief processing flow diagram is shown in Figure 2. After every processing is finished, both
**Table:**

| Compound        | MW  | Log Kow | Water Solubility | Vapor Pressure |
|-----------------|-----|---------|------------------|----------------|
| Hexachlorobenzene | 285 | 5.73    | 0.006mg/L        | 0.00018mmHg    |
| Pentachloronitrobenzene | 295 | 4.64    | 0.44mg/L         | 0.00005mmHg    |
| α-BHC           | 291 | 4.14    | 8mg/L            | 0.0000352mmHg  |
| γ-BHC           |     |         |                  |                |
| δ-BHC           |     |         |                  |                |

**Figure 1:** Structures and properties of (a) hexachlorobenzene, (b) pentachloronitrobenzene, (c) α-BHC, (d) γ-BHC, and (e) δ-BHC. Referential pesticide properties are at http://www.chemspider.com/Default.aspx (accessed 24 October 2019).

**Figure 2:** Ginseng processing flow diagram, sample steps, and pesticide concentration in each critical stage. Values correspond to the average of three replications.
the products and dregs were stored in a hermetic container, respectively, at −20°C, and the pesticide residues were determined in one day.

2.3.1. Boiling. Five grams of raw ginseng pieces were soaked in a heating mantle with 100 mL of distilled water for 30 min, heated to boil with high flame, simmered to low flame for 30 min, allowed to cool, and filtered. The dregs were taken, and the procedure above was repeated again. The filtrates were merged as ginseng decoction, and the dregs were allowed to air-dry.

2.3.2. Brewing. Five grams of raw ginseng pieces were brewed with 150 mL of boiling distilled water, allowed to cool, and filtered. The dregs were taken, and the procedure above was repeated again. The filtrates were merged as ginseng infusion, and the dregs were allowed to air-dry.

2.3.3. Ethanol Refluxing. Five grams of raw ginseng pieces were put in a round-bottom flask with 80 mL of 80% ethanol, heated to reflux in an 80°C water bath for one hour, allowed to cool, and filtered. ©Q_ The dregs were taken, and the procedure above was repeated again. The filtrates were merged as ginseng decoction, and the dregs were allowed to air-dry.

2.3.4. Boiling Combined with Resin Purification. Five grams of raw ginseng pieces were boiled in a heating mantle with 100 mL of distilled water for two hours, allowed to cool, and filtered. The dregs were taken, and the procedure above was repeated again. The filtrates were merged, concentrated, and dried as the dried ethanol extract, and the dregs were allowed to air-dry.

2.4. Pesticide Analysis. The analysis of the OCPs in liquid samples was performed based on Determination of Organochlorine Pesticide Multiresidues in Foods (standard GB/T5009.19–2008) [25]. The analysis of the OCPs in solid samples was performed based on the methods developed by our laboratory previously [26, 27].

2.5. Sample Extraction and Cleanup

(1) Liquid samples (40 ml of decoction and infusion were taken for the following treatment, respectively; the dried ethanol extract and total ginsenoside were dissolved in 40 ml distilled water, respectively): 40 mL of each liquid sample was extracted by 20, 20, and 20 mL of dichloromethane successively, the dichloromethane phase was merged, and the extra water in the dichloromethane phase was removed by anhydrous sodium sulfate. The dichloromethane extracts were then concentrated to a few milliliters by a rotary vacuum evaporator at 40°C.

(2) Solid samples (raw ginseng and dregs of ginseng): each solid sample was ground mechanically to homogeneous powder and sieved through a No. 24 mesh sieve (850 ± 29 mm aperture). 2 g of powder was sonicated in an ultrasonic bath for 15 min with 30 mL of acetone/petroleum ether (1:1, v/v) and then filtered. The extract procedure was repeated again with additional 20 mL of acetone/petroleum ether (1:1, v/v). The extracts were merged and concentrated to a few milliliters by a rotary vacuum evaporator at 40°C.

(3) Concentrated solution of both liquid and solid samples: this was transferred into a 5 mL test tube with the calibrated scale and diluted to 3 ml with petroleum ether precisely; then, 1 mL of sulfuric acid/water (90:10, v/v) was added, and the solution was shaken vigorously for 1 min using a vortex mixer. The mixture was centrifuged at 3000 r/min for 10 min to separate two layers. An aliquot of the upper organic layer was moved into another test tube. Then, 3 mL of distilled water was added, and the solution was shaken vigorously (to remove the acid). The mixture was centrifuged at 3000 r/min for 10 min again to separate two layers. An aliquot of the upper organic layer was moved into another test tube, and 2 g of anhydrous sodium sulfate was added to remove the water. Finally, an aliquot of the upper organic layer (about 1 mL) was transferred into a GC vial for GC-ECD and/or GC-MS/MS analysis.

2.6. Instrument Analysis

2.6.1. GC-ECD Analysis. The column temperature was 120°C at the start, held for 1 min; increased to 150°C at a rate of 8°C/min, held for 2 min; and then increased to 270°C at 4°C/min, maintained for 7 min. The temperature of the injector was maintained at 250°C in the splitless mode. The detector temperature was maintained at 300°C. High-purity nitrogen (N₂, 99.999%) was used as the carrier gas with a flow rate of 1 mL/min. Injection volume of the sample was 1 μL, and the content of every pesticide in all samples was calculated by the external standard method.

2.6.2. GC-MS/MS Analysis. The injector temperature was maintained at 250°C in the splitless mode, and the volume of the injection was 1 μL. High-purity helium (He, 99.999%) was used as the carrier gas with a constant flow of 1 mL/min. The column temperature was 40°C at the start, held for 1 min; increased to 120°C at a rate of 30°C/min; increased to 180°C at 20°C/min, and increased to 280°C at 15°C/min, maintained for 10 min. Table 1 lists the determination parameters of five pesticides by GC-MS/MS, including the parameters of the tandem MS system, the retention times, the qualification and quantification ions, and the collision energies for each pesticide.
Tandem MS detection Ionisation mode EI Electron energy 70 eV SIM width 0.7 amu Transfer line temperature 280°C Ion source temperature 230°C Collision gas Ar (99.99%) CID pressure 2.0 mTorr

| Pesticide                  | Retention time (RT, min) | Qualification ions [Q1 → Q3 (CE), m/z → m/z (V)] | Quantification ions |
|----------------------------|--------------------------|--------------------------------------------------|--------------------|
| α-BHC                      | 10.85                    | 219 → 181 (10)                                   | 181 → 145 (15)     |
| Hexachlorobenzene (HCB)    | 11.54                    | 284 → 249 (30)                                   | 284 → 214 (35)     |
| Pentachloronitrobenzene (PCNB) | 11.68            | 237 → 143 (25)                                   | 295 → 237 (20)     |
| γ-BHC                      | 11.86                    | 183 → 147 (15)                                   | 181 → 145 (15)     |
| δ-BHC                      | 12.85                    | 217 → 145 (15)                                   | 181 → 145 (15)     |

Table 1: Parameters for the determination of five pesticides by GC-MS/MS.

2.7. Method Validation. The quantitative determination of five OCPs in all samples was done by GC-ECD on the basis of an external standard method. The calibration curves were obtained by injecting five different concentrations of the pesticide standards in the range of 0.001–0.2 mg·L⁻¹. For the recovery and precision, 40 mL of ginseng infusion was spiked with 0.5 mL of 0.2 mg·L⁻¹ of each pesticide (spiked concentration level: 2.5 µg·L⁻¹) in three replicates; 2 g of raw ginseng powder was spiked with 0.5 mL of 0.4 mg·L⁻¹ of each pesticide (spiked concentration level: 0.1 mg·kg⁻¹) in three replicates for overnight absorption. The pesticide analysis procedure was conducted as liquid and solid samples, respectively. As shown in Table 2, linearity of five pesticides procedure was conducted as liquid and solid samples, respectively. As shown in Table 2, linearity of five pesticides was good with the correlation coefficients (r²) over 0.999. Recoveries of all the pesticides ranged from 70.3% to 85.6% and 83.4% to 106.9% in liquid and solid samples, respectively. The LOQs ranged from 0.02 to 0.12 µg/L in liquid samples and 0.001 to 0.004 µg/kg in solid samples.

2.8. Statistical Analysis. The statistical software package SPSS 17.0 was used for data analysis. The transfer rates of pesticides studied in the present study were calculated by the following equation:

\[
\text{transfer rate (\%)} = \frac{C_{\text{product}} \times X_{\text{product}}}{C_{\text{ginseng}} \times M_{\text{ginseng}}} \times 100\%.
\] (1)

where \(C_{\text{product}}\) and \(C_{\text{ginseng}}\) refer to the concentration of pesticides in the ginseng product and raw ginseng, respectively; \(X_{\text{product}}\) is the volume or weight of the ginseng product; and \(M_{\text{ginseng}}\) is the weight of raw ginseng.

3. Results and Discussion

The study of the fate of OCPs during household and industrial processing of ginseng allows for calculating the transfer rates of OCPs. These transfer rates provide vital reference to the risk assessment of frequently detected OCPs.

3.1. Raw Ginseng. The confirmation of targeted pesticides was done by GC-MS/MS. Gas chromatography-tandem mass chromatograms of detected pesticides in the raw ginseng sample and standard pesticide mixture are shown in Figure 3. As shown in Figure 2, the average contents of hexachlorobenzene (HCB), pentachloronitrobenzene (PCNB), α-BHC, γ-BHC, and δ-BHC in raw ginseng were 0.282, 3.057, 0.513, 0.029, and 0.086 mg/kg, respectively. Consistent with our findings, Ying et al. [28] detected 246 pesticide residues in 80 ginseng samples collected from different places in China by GC-MS/MS and LC-MS/MS methods, and they found that detection rates and average contents of HCB, PCNB, and total BHC were 61.2%, 77.8%, and 3.8%, respectively, and 0.230, 3.141, and 0.068 mg/kg, respectively. Therefore, the OCPs in ginseng we studied were representative. The content of PCNB was evidently higher than that of other pesticides because PCNB is still used, while HCB and BHC have been banned in China. However, the content of HCB was relatively high, and it is possible that HCB is a by-product in the production of PCNB [29].

3.2. Household Processing. The products of ginseng household processing (boiling and brewing) were decoction and infusion. The average contents and transfer rates of pesticide residues are presented in Figures 2 and 4. The transfer rates of HCB and PCNB from raw ginseng to decoction were 3.44% and 3.09% and to infusion were 1.47% and 2.37%, respectively. The transfer rates of α-BHC, γ-BHC, and δ-BHC from raw ginseng to decoction were 24.83% to 34.43% and to infusion were 26.59% to 38.12%, respectively. The transfer rates of HCB and PCNB were lower than those of BHC in both boiling and brewing probably because the water solubility (Figure 1) of HCB (0.44 mg/L) and PCNB (0.006 mg/L) is lower than that of BHC (8 mg/L). As shown in Figure 1, organochlorine pesticides are hydrophobic substances with high log Kow (octanol/water partition coefficient) values. However, the results of transfer rates of BHC during ginseng boiling and brewing were unexpectedly high. Chen and Wan [30] and Manikandan et al. [18] found that the transfer rates of organochlorine pesticides including BHC, endosulfan, DDT, and dicofol were less than 7% during tea brewing. Additionally, Wan et al. [31] demonstrated that the transfer rates of pesticide residues were 1–4%
Table 2: Results of the method validation study, including retention time, correlation coefficients, LOQs, and recoveries.

| Pesticide                      | RT (min) | Correlation coefficients ($r^2$) | Liquid sample (μg/L)  | Solid sample (mg/kg) | Recovery and precision ($n = 3$) |
|--------------------------------|----------|----------------------------------|-----------------------|----------------------|---------------------------------|
|                                |          |                                  | LOQ (S/N = 10)        | LOD (S/N = 3)        | Average (%) ± SD                |
| Hexachlorobenzene (HCB)        | 4.83     | 1                                | 0.05                  | 0.02                 | 0.002 ± 0.0006                   | 70.3 ± 2.7 ± 83.4 ± 10.7       |
| α-BHC                          | 5.34     | 0.9999                           | 0.02                  | 0.01                 | 0.002 ± 0.0006                   | 85.6 ± 5.3 ± 91.9 ± 10.9       |
| Pentachloronitrobenzene (PCNB) | 5.54     | 0.9998                           | 0.08                  | 0.02                 | 0.004 ± 0.0012                   | 79.4 ± 5.7 ± 97.1 ± 14.9       |
| γ-BHC                          | 5.90     | 0.9999                           | 0.02                  | 0.01                 | 0.001 ± 0.0003                   | 71.5 ± 4.0 ± 106.9 ± 11.6      |
| δ-BHC                          | 7.34     | 0.9999                           | 0.12                  | 0.04                 | 0.002 ± 0.0006                   | 82.1 ± 6.0 ± 83.9 ± 4.7        |

Figure 3: Gas chromatography-tandem mass chromatograms of (a) detected pesticides in the raw ginseng sample and (b) standard pesticide mixtures. Peaks: 1, α-BHC; 2, hexachlorobenzene (HCB); 3, pentachloronitrobenzene (PCNB); 4, γ-BHC; 5, δ-BHC.

Traditionally, ginseng is considered a tonifying and nourishing herb, which should be boiling longer to extract more active ingredients. People may worry whether the pesticide residues will be extracted together in the wake of long-time boiling. However, in Figure 4(a), it is worth noting that the transfer rates of five pesticides in boiling (boiling for two hours in total) were similar to those in brewing (without boiling procedure). It indicates that the transfer rates of the OCPs in this study will not increase significantly within two minutes.
hours of boiling. Similarly, Cho et al. [34] and Jiaying et al. [35] found that water temperature resulted in small changes in the transfer rates of some pesticides like azoxystrobin and EPN (ethyl p-nitrophenyl). Besides, in our study, the remaining of pesticides in ginseng dregs obtained by boiling was approximately half less than that in ginseng dregs obtained by brewing, except for γ-BHC and δ-BHC (Figure 4(b)). This may be due to the low water solubility (all < 10 mg/L) of these pesticides, and they were vaporized to the air or decomposed during boiling, rather than transfer to water [36].

3.3. Industrial Processing. The products of ginseng industrial processing (ethanol refluxing and boiling combined with resin purification) were the dried ethanol extract and total ginsenosides. The average contents and transfer rates of pesticide residues are presented in Figures 2 and 4. The average concentration of hexachlorobenzene (HCB), pentachloronitrobenzene (PCNB), α-BHC, γ-BHC, and δ-BHC in the dried ethanol extract was 0.307, 4.205, 0.962, 0.092, and 0.275 mg/kg, respectively, all higher than that in raw ginseng. It reveals that the OCPs were concentrated during the ethanol refluxing procedure. The transfer rates of OCPs during the ethanol refluxing procedure range from 34.81 to 67.27%; it means that the transfer rates of this processing method were highest in four processing methods. These results can be explained by the high log Kow values of OCPs (Figure 1), and the weight of the dried ethanol extract was less than that of the raw ginseng (Figure 2). Therefore, the raw ginseng materials containing high magnitude of OCPs are discouraged to employ in ethanol extraction. The contents of OCPs in total ginsenosides were almost lower than LODs (Figure 2); this is possibly because the pesticides were absorbed by the resin. It indicates that boiling combined with resin purification can purify ginsenosides and remove OCPs at the same time. Consequently, to make full use of the ginseng resource, this method is encouraged to deal with those unqualified raw ginseng materials in excess of the maximum residue limits (MRLs) of OCPs.

3.4. Ginseng Dregs Obtained by Processing. In general, the dregs of ginseng obtained by processing may be used as animal feed and organic fertilizer [37]. Therefore, we should also pay attention to the pesticide residues in ginseng dregs. As illustrated in Figure 4(b), the OCPs in brewing ginseng dregs were highest ranging from 60.40 to 72.69%, next to those in the boiling ginseng dregs ranging from 22.38 to 62.20%; the dregs obtained by ethanol refluxing were lowest and almost lower than LODs (Figure 4(b)). It means that the dregs obtained by ethanol refluxing can be used more safely, while the dregs obtained by boiling and brewing have the risk of pesticide residues.

4. Conclusion

The transfer rates of OCPs during household and industrial processing were variable, which are related to the pesticides’ physicochemical properties, such as water solubility and log Kow. Before processing, the contents of pesticide residues in raw ginseng were high, while the pesticide residues decreased differently after processing: the boiling combined with resin purification method removed the OCPs most effectively; the OCPs were concentrated during the ethanol refluxing procedure; the transfer rates of the OCPs in water extraction were relatively low and would not increase significantly along with two hours of boiling.

Data Availability

All data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this manuscript.


Authors’ Contributions

All authors read and approved the final version of this article to be published.

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