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Distribution of Iodine^{125} Labeled Parathion and the Protective Effect of Dried Banana Peel in Experimental Mice

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Abstract

Parathion (PA) one of the organophosphorus pesticides, is widespread in agriculture crops. Several studies reported the detection of this pesticide residue in food at high concentrations. Accordingly, the purpose of this study was to determine the accumulation of iodine [^{125}I] labeled parathion ([^{125}I] PA) in mice organs and to study the protecting reflex of dried banana peel. In this study, two groups of mice were used. Iodine [^{125}I] labeled parathion (10 mg/kg body weight) was applied only to one group of mice. Meanwhile, [^{125}I] PA (10 mg/kg body weight) and dried banana peel (20% were added to the diet) were applied to the other group of mice. The mice were injected by [^{125}I] PA through the tail vein and were given the dried banana peel through the mouth. The mice were kept under observation for 180 min. for monitoring. The result of the first group of mice, which was injected with [^{125}I] PA, only indicated that [^{125}I] PA was distributed primarily in the kidneys (18.6%), liver (18.9%), and intestine (6.11%), after 180 min. In the second group of mice which received both [^{125}I] PA, and dried banana peels, [^{125}I] PA accumulated at a lower percentage in the kidneys (10.2%), liver (5.2%), and intestine (4.67%) after 180 min. These results indicated the decrease of [^{125}I] PA in the liver and kidneys could be due to the effect of dried banana peels, and draw attention to the use of agricultural waste to remove pesticide residues contaminating food and feed.

***Keywords*:** dried banana peels; parathion; [^{125}I] PA; biodistribution; organs; mice.

1. Introduction

Parathion is an organic thiophosphate, a C-nitro compound, and an organothiophosphate insecticide (Figure 1). Worldwide crops are exposed to approximately 2.5 million tons of pesticides each year. The agro-systems are one of the most important food contaminants that threaten the safety of food [1]. In recent years scientists and the public are concerned about the environmental issues induced by the overuse of pesticides. It is reported that the crop loss due to pest infestation can be as high as 100% if they are not controlled, whereas damage caused by pesticides reaches about $100 billion annually [2].

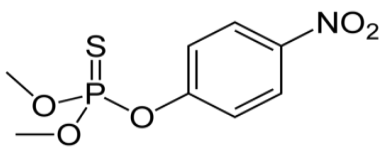

Figure (1): The chemical structure of parathion

The damage caused by pesticides is due to the high toxicity and non-biodegradable properties of pesticides and also due to the presence of pesticide residues in soil, water resources, and crops that affect public health. Consumption of food containing pesticide residues is the major route of chronic exposure to pesticides which may cause hazards to human health [3, 4]. The WHO reported that about three million people are poisoned by pesticides and that 220,000 deaths happen in developing countries every year [5, 6]. Pesticides have consequent long-term adverse impacts on the national income, ecological system, and public health [7].In a previous study in Egypt, pesticide residues were detected in various foods such as vegetables and cereals [8]. Agricultural waste peel is a natural, ecological and economical source of adsorbent which can be used to remove various pollutants and reduce pollution, and in the last ten years, several methods and approaches have been improved to re-use fruit and vegetable waste [9]. Banana, Musa spp., is a tropical fruit

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consumed globally, including multiple varieties, whereas its peel is mainly used for the production of compost, animal feed and protein, ethanol, methane, pectin, and enzymes [10]. Banana peel is rich in phytochemical compounds, generally, antioxidants, and their potential application depends on its chemical composition [11]. It is rich in proteins (7% DW), dietary fiber (50% DW), polyunsaturated fatty acids, potassium, and essential amino acids [12]. Banana peel contains large amounts of L-dopa, catecholamines, and dopamine with a significant antioxidant activity [11], and has also been used as food for livestock [13], and/or as an adsorbent for water purification [14]. Compounds like anthocyanin, delphinidin, and cyaniding are also found in ripe banana peels [15]. Iodine^{125} is the perfect iodine isotope due to its appealing half-life (59 days), X-ray emissions (27–35 keV), and low energy gamma, thus making it suitable for radioactive detection with low exposure to the surrounding tissue [16]. The ^{125}I is primarily used for in vitro research, and as all iodine radioisotopes, they have the same biodistribution profile and give the same chemical properties [17]. The capacities of these strategies for radiotherapy and/or imaging are increased with other iodine radioisotopes [18]. Accordingly, the purpose of this study was to determine the accumulation of iodine [^{125}I] labeled parathion in mice organs and to study the protecting reflex of dried banana peel.

2. Materials and Methods

2.1. Chemicals and reagents

No-carrier-added (NCA) Na125I (185 MBq/50 µL) diluted in 0.04M NaOH, pH 9-11 was purchased from the Institute of Isotopes, Budapest, Hungary. Parathion was purchased from Santa Cruz Biotechnology Inc., (Santa Cruz, CA 95060, USA). Chloramine-T [N-chloro-p-toluene sulfonamide salt] (CAT), acetonitrile, ethanol, methanol, methylene chloride, ethyl acetate, and citric acid were purchased from Sigma-Aldrich (Illinois, USA). Thin layer chromatography (TLC) aluminum sheets (20 cm x 25 cm) SG-60 F254 was purchased from Merck (Darmstadt, Germany). Whatman filter paper No. 1 (Maid Stone, Kent, UK) was used. The buffer was composed of 4.7 g of sodium dihydrogen orthophosphate and 1 mL of triethylamine in 1000 mL of water and the pH of the solution was adjusted to 4.0 with orthophosphoric acid. All chemicals were of clinical grade and were used directly without further purification unless otherwise stated. Double distilled water was used in all experiments for the preparation of solutions, dilution, and washing purposes.

2.2. Optimization of reaction

In an amber colored vial radioiodination conditions were scrutinized and optimized to maximize the yield of the labeled compound by using CAT (25-200µg) of freshly prepared solutions, dissolved in ethanol (1:1 v/v), different concentrations of PA (10-100 µg) dissolved in ethanol (1:1 v/v), pH (3-9), reaction time (5-120 min), and stability [19, 20].

2.3. Radiolabeling procedure

In the presence of CAT as an oxidizing agent, NCA 125I [10 µl (7.50 MBq)] was used to synthesize iodine-labeled parathion by direct electron radioactive iodination. The volume of the reaction mixture was set to ~550 µl. Radio iodination conditions were scrutinized and optimized to maximize the yield of the labeled compound in an amber-colored vial. Parathion (50µg) was dissolved in ethanol (1:1 v/v). No-carrier-added Na125I was transferred to the reaction system and evaporated to dryness by vacuum. Chloramine T (100µl) was added to the reaction flask. The reaction mixture was stirred with a magnetic stirrer (Model 210 T Thermax, Fisher, USA) and left at 400°C for 15 min. Then 100 µl of sodium metabisulphite (60 mg/mL) was added to decompose the excess of iodine to stop the reaction, and the radiotracer was isolated. Then, the percent radiochemical yield was determined using TLC, electrophoresis (E.C. Corporation, Albany, OR, USA), and completely purified using HPLC (SHIMADZU Cooperation, MD, USA) [21, 22]. The Shimadzu model detector SpD-6A consists of pumps LC-9A, column Lichrosorb RP-C18 (250 mm x 4.6 mm), 5 lm, rheodyne injector, UV spectrophotometer detector at 250 nm wavelength, and a mobile phase constituting of buffer and acetonitrile (70:30 % v/v).

2.4. Radiochemical analysis of iodine labeled parathion

TLC method was employed to determine the radiochemical yield of the [^{125}I] PA. A volume of 5 µL (3.20 MBq) reaction mixtures was placed on the start line and then eluted using methylene chloride: ethyl acetate (2:1 v/v) as a developing system. After complete development, the strips were removed, dried and cut into 1 cm segments and assayed for radioactivity using NaI scintillation γ-Counter (model Scalar Ratemeter SR7, Nuclear Enterprises Ltd., USA) [23] The radiochemical yield was further confirmed by paper electrophoresis using Whatman filter paper No. 1 (2cm width and 47cm length), 2–5µl of the reaction mixture was placed at 12cm far from the negative electrode edge of the paper sheet. Electrophoresis was carried out for 1.5 hours at a voltage of 300 volts using normal saline (0.9% w/v NaCl solution), pH 7 as electrolytes solution. When

the development was completed, then the paper was removed, dried, and cut into strips, each strip of 1cm width. The strip was then counted in a NaI scintillation γ-Counter (model Scalar Ratemeter SR7, Nuclear Enterprises Ltd., USA). The percentage of radiochemical yield was calculated as the ratio of the radioactivity of [^{125}I] PA to the total activity multiplied by 100 [24] High performance liquid chromatography analysis was finally used for purification of [^{125}I] PA by direct injection of 15 µl of the reaction mixture into the column mentioned above using a previous mobile phase. The flow rate was at 1.0 mL/min at ambient temperature. Fractions of 1 mL were collected separately using a fraction collector up to 15 mL by using a single-channel analyzer.

2.5. Preparation dried banana peel

Bananas were obtained from fruit retailers, Cairo, Egypt, and were thoroughly cleaned by washing under tap water, and then soaked in double-distilled water for 1 h to remove any dust or dirt that adheres to the banana peel, and then wiped dry. Subsequently, the banana peel was removed manually and the pulp is extracted. Then the banana peels were soaked in 1% citric acid solution for 12 h to prevent oxidation and enzymatic browning. After that, the banana peels were dried at 50°C for 96 h, and were ground and passed through a 0.25 mm mesh. Finally, the ground powder was thereafter stored in clean brown bottles at room temperature [25]. Analysis of the functional groups present in the dried banana peel was carried out by absorption spectroscopy in the infrared region (400-4000 cm^{-1}) [26]

2.6. Experimental animals

Fifty female BALB/c mice weighing between 30 and 40 grams were obtained from the Animal House Colony of the National Research Center. The animals were allowed to be acclimatized at room temperature under a 12 hour light/dark cycle and 70% relative humidity. After a one-week acclimation period, the animals were divided into two groups (25 mice/group), whereas the first group was treated intravenously with iodine [^{125}I] labeled parathion 0.2 mL (3.6 MBq) (10mg/kg body weight) adjusted to physiological pH via the tail vein [27], and the second group was treated intravenously with [^{125}I] PA (10mg/kg body weight) and fed on a dried banana peel (20% of diet) for two weeks before labeling with 0.2 mL (3.6 MBq) [^{125}I] PA. The mice were kept in a polycarbonate cage with a filter cover. All animals received human care in agreement with the guidelines of the Animal Care and Use Committee of the National Research Center, Dokki, Cairo, Egypt, and the National Institutes of Health (NIH publication 86-23 revised 1985). The experiment was also approved by the ethics committee of the labeled compound Department.

2.7. Biodistribution of iodine labeled parathion

At different times after injection (15, 30, 60, 120, and 180 minutes), five mice were sacrificed by cervical dislocation under chloroform anesthesia. All major organs were separated and measured by comparison with a standard solution of the labeled substrate. Fresh blood was collected from the retro-orbital plexuses in mice, weighed and radioactivity was measured using NaI scintillation γ-Counter (model Scalar Ratemeter SR7, Nuclear Enterprises Ltd., USA). Bone and tissue samples were also collected washed, weighed and radioactivity was measured using NaI scintillation γ-Counter The mean percentage of the administrated dose/per gram was calculated. Assumption of the proportions of blood, bone, and muscle were 7%, 10%, and 40% of total body weight, respectively [28]. During the experiment, the background radiation and decay were corrected.

2.8. Stability of radioiodinated olmesartan in serum

Normal rat serum (1.9 mL) was mixed together with [125I] PA (0.1 mL [0.15 MBq]) and left at room temperature for 24 h. After the incubation period, about 0.2 mL was withdrawn for the determination of the purity of the radiochemical labeled compound using HPLC (SHIMADZU Cooperation, MD, USA) and counted in a well-type NaI scintillation γ-Counter (model Scalar Ratemeter SR7, Nuclear Enterprises Ltd., USA) [29].

2.9. Statistical analysis

Data were expressed as mean ± SE (n=3). Statistical analysis of the data was carried out using Microsoft Excel 2010 statistical program. A one-way analysis of variance (ANOVA) was performed, in which $P < 0.05$ was considered statistically significant. Fisher's Protected Least Significant Difference was also used to determine the difference between different means [30].

3. Results and Discussion

3.1. Evaluation of radiochemical yield by TLC, paper electrophoresis, and HPLC

The results showed that the retention factor (Rf) for free iodide ranged from 0.0 to 0.1, whereas the Rf for the [^{125}I] PA ranged from 0.8 to 1.0. In

addition, paper electrophoresis was used in which the free iodide and iodine [^{125}I] labeled parathion proceeded to diverse distances to the positive electrode away from the spotting point, whereas the distance from the spotting point recorded 12 and 0 cm respectively. For more clarification HPLC was used, in which the retention time (Rt) of free iodide and [^{125}I] PA were after 3.5 and 5 min, respectively as shown in (Figure 2), whereas UV of olmesartan medoxomil was after 3.5 min. [31-33].

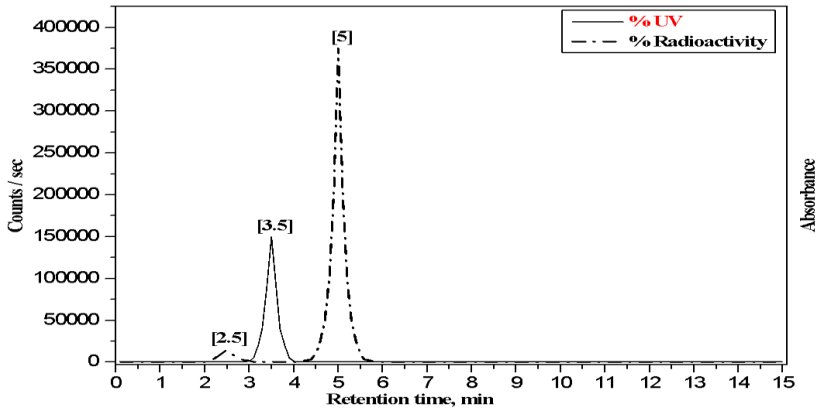

Figure (2): HPLC radio chromatogram of co-injection of iodine labeled parathion and UV of parathion.

3.2. Optimization of reaction

Each element was checked separately, keeping other elements regular, such as the pH value of the reaction mixture, the amount of oxidizing agent, the amount of ligand, and the reaction time of the mixture, that are optimized at room temperature. No doubt the substrate amount of PA affected the radiochemical yield of the labeled compound. Data in Figure (3) showed the effect of different pH on the variation of the radiochemical yield, whereas different pH values ranging from pH 3.0 to 9.0 were used. Results revealed that the percentage of the radiochemical yield increased by increasing the pH value from 3.0 up to 6.0, whereas the maximum yield percentage recorded 98.5% at pH 6.0. It was also observed that any increase in pH above 6.0 resulted in a decrease in the radiochemical yield percentage, which recorded 55.0% at pH 9. An increase in pH above 6 (very alkaline) may have resulted in the formation of hypo iodide (IO-) ions, which could have been converted to iodate (IO^-_3) and iodide ions (I^-). Chloramine T acts as an oxidizing agent in both acidic and alkaline media [34]. Chloramine T acts as a strong electrolyte and produces different reactive species in the solution based on the pH of the medium [35]. Chloramine T is hydrolyzed in an acidic medium to form hypohalous acid (HOCl), which will be further hydrolyzed to form H_2OCl^+, so the possible oxidizing agents in acidified CAT solution are HOCl and H_2OCl^+, while the alkaline CAT solution is HOCl and ClO [36]. Therefore, it reacts quickly with olmesartan at any site where it can enter the electrophilic substitution reaction [37-40].

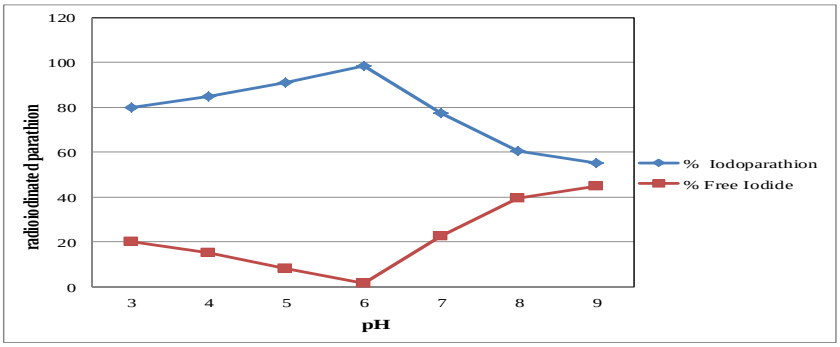

Figure (3): Effect of different pH on the variation of the radiochemical yield. Results showed significant difference P<0.05.

Results in Figure (4) showed the effect of different concentrations of parathion on the variation of the radiochemical yield, whereas different concentrations ranging from 10 to 100µg. Results revealed that the optimum radiochemical yield was obtained when using 50 µg, whereas radiolabeled compound recorded 98.50%. Data also indicated that radiolabeled compound was not affected by increasing the concentration to 100µg.

Data in Figure (5) revealed the effect of different concentrations of the oxidizing agent CAT on the variation of the radiochemical yield, whereas CAT concentrations used ranged from 25 to 200µg. Results indicated that radiolabeled compound increased in the presence of CAT at a concentration of 100µg (µl), giving an optimum radiochemical conversation at 98.5%. Excessive CAT can lead to the formation of unwanted oxidation by-products [41]. Figure (6) exhibited the effect of reaction times on the variation of the radiochemical yield, whereas different treatment times ranged from 5 to 120 min. The radiolabeled compound reached optimum after 15 min. On the other hand, data in Figure (7) displayed the effect of stability during 48 h on the variation of the radiochemical yield. Results revealed that the radiolabeled compound reached 98% after 3 h. After 12 h radiolabeled compound reached 96% and then decreased to 93% after 48 h. These results indicated that the radiolabeled compound decreased by increasing the treatment time [42].

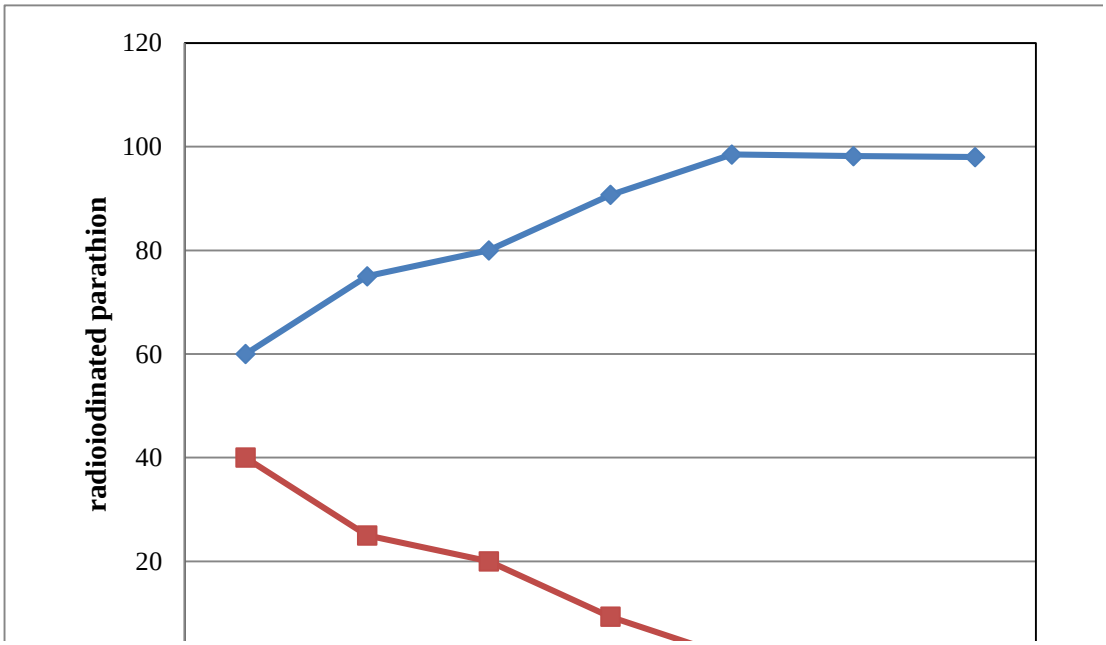

Figure (4): Effect of different concentrations of parathion on the variation of the radiochemical yield. Results showed significant difference $P<0.05$

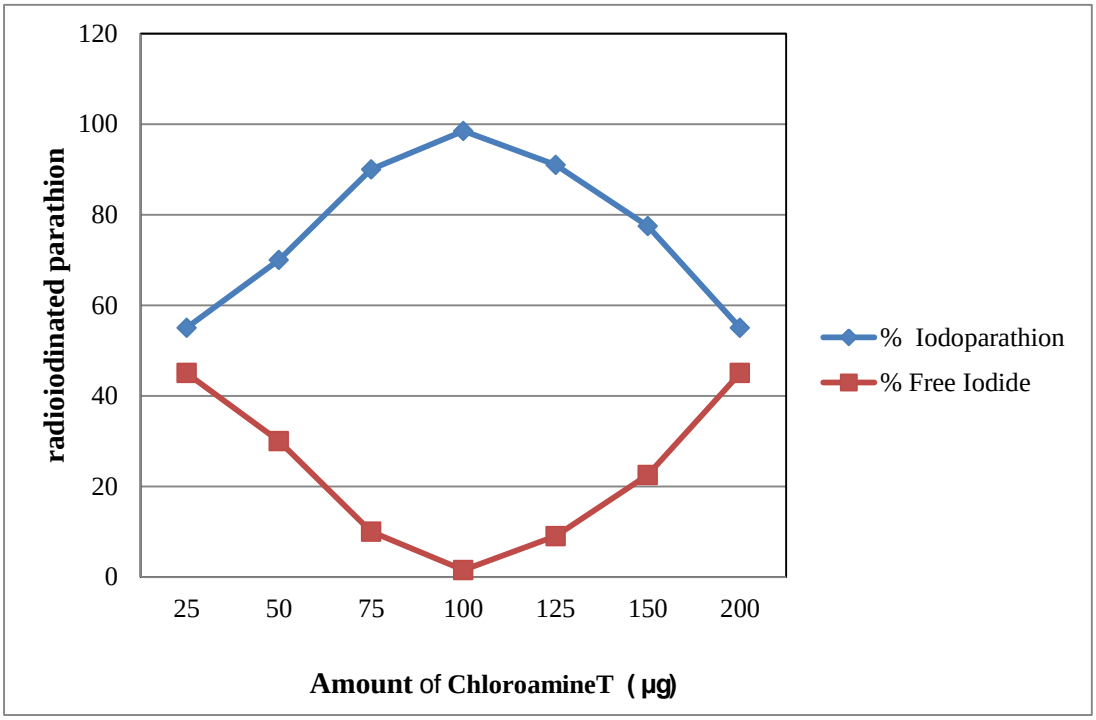

Figure (5): Effect of different concentrations of chloramine T on the variation of the radiochemical yield. Results showed significant difference $P<0.05$

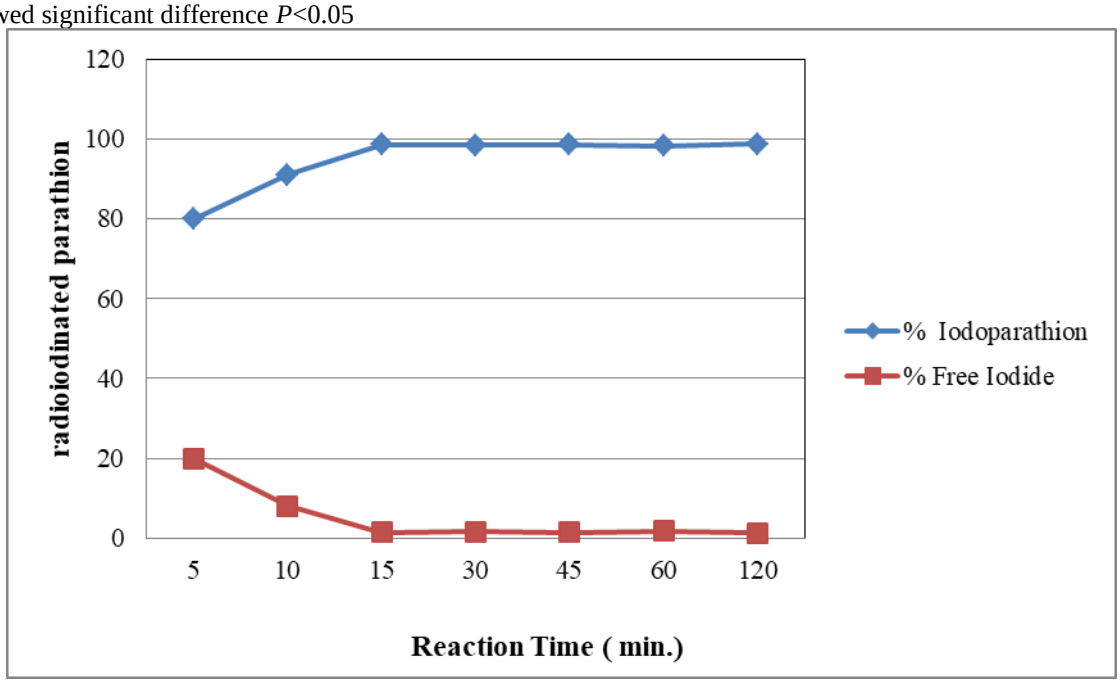

Figure (6): Effect of different reaction times on the variation of the radiochemical yield. Results showed no significant difference $P>0.05$

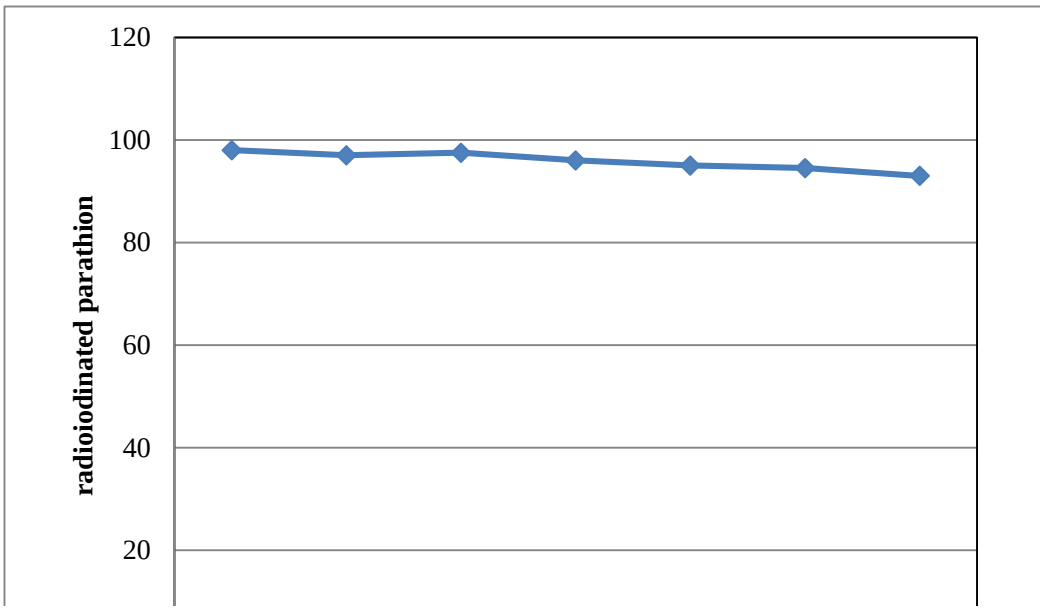

Figure (7): Effect of stability during 48 h on the variation of the radiochemical yield. Results showed no significant difference $P>0.05$

3.3. Characterization of banana peel

Fourier Transform Infrared spectra of banana peels were determined to understand the nature of the functional groups present. Data in Figure (8) displayed several peaks for the banana peels. Bands appeared at 3432.67, 2926.45, 1630.5, 1421.28, 1054.87, and 620.002 cm^{-1} and were assigned to O-H stretching, C-H stretching of alkane (stretching of carboxylic acid or ester), C=C stretching of alkene, and -C-H bending of alkane, respectively. Therefore, the FTIR spectrum profile established the occurrence of carboxylic acid, alcohol, alkanes, alkenes, and amines. Similar results were reported by Pathak et al. [43]. Out of these functional groups, carboxylic acid and hydroxyl groups may have played a major role in the removal of parathion [44]. The main source of carboxylic acid in fruit peel could be cellulose, pectin, or lignin [45]. On the other hand, the FTIR did not show any peaks between 2220 and 2260 cm^{-1}, thus suggesting the absence of cyanide groups, and confirming that banana peel does not contain any toxic substances [46].

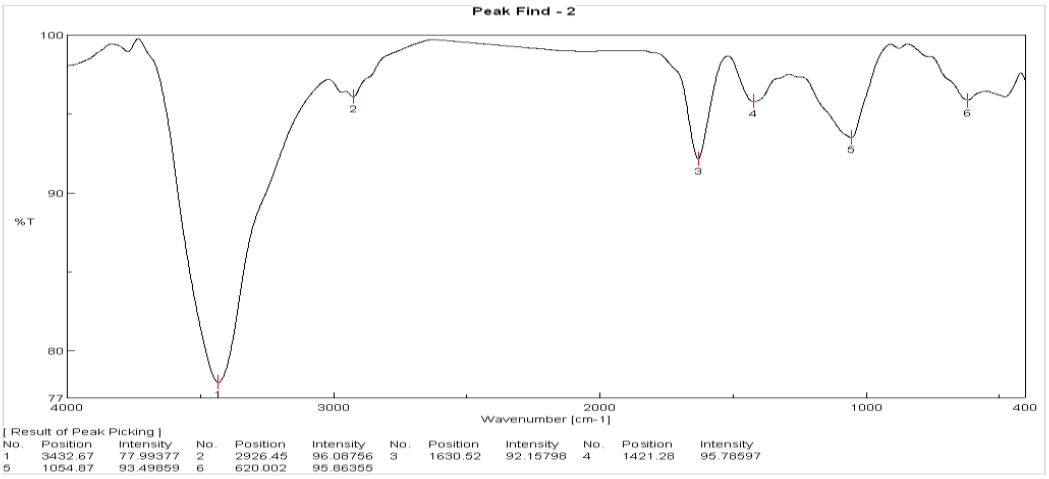

Figure (8): FTIR spectra of banana peel

3.4. Biodistribution of [^{125}I] PA

The data in Figure (9) showed the biodistribution of [^{125}I] PA in various body organs and fluids. Most radioactivity values were signified as an average percentage of the administered dose per gram of tissue (% ID/g). Iodine labeled parathion accumulated rapidly in the liver (7.28%), kidney (6.22%), blood (5.11%), and the intestine (1.59%) after 15 min. of injection. A similar study revealed that parathion distributes primarily to the liver, whereas lower levels were detected in other organs including the kidney [47]. The accumulation in the liver and kidney increased up to 25.11% and 28.60% respectively after 120 min and decreased to 18.9% and 18.6% respectively after 180 min. This decrease may indicate that the radiolabeled compound is excreted through hepatobiliary and urinary pathways [48]. Results also revealed that [^{125}I] PA was detected in blood (5.11%) after 15 min. and decreased to reach 0.19% after 180 min.

3.5. Effect of the dried banana peel on the distribution of [^{125}I] PA

Data in Figure (10) showed the biodistribution of [^{125}I] PA in mice that were previously given banana peel, as it is essential to compare the accumulation of [^{125}I] PA in both groups. Iodine labeled parathion was detected in kidney (3.l9%), liver (2.21%), and intestine (1.51%) after 15 min. These results were considered to be less than those of the control mice which received [^{125}I] PA only, hence signifying that banana peels can reduce the accumulation of [^{125}I] PA in organs.

In this study, banana peel proved to be a suitable bio adsorbent for removing parathion, as it has a high adsorption capacity [10]. The high adsorption capacity of banana peels could also be due to the presence of the functional groups O-H, C-H, and C=C, which provided appropriate surface functions for the interaction with contaminants [49]. Therefore, this study point out that bio adsorbents are easily obtained from biomass that is generally considered as waste, making it less expensive, especially when implanting at a large scale.

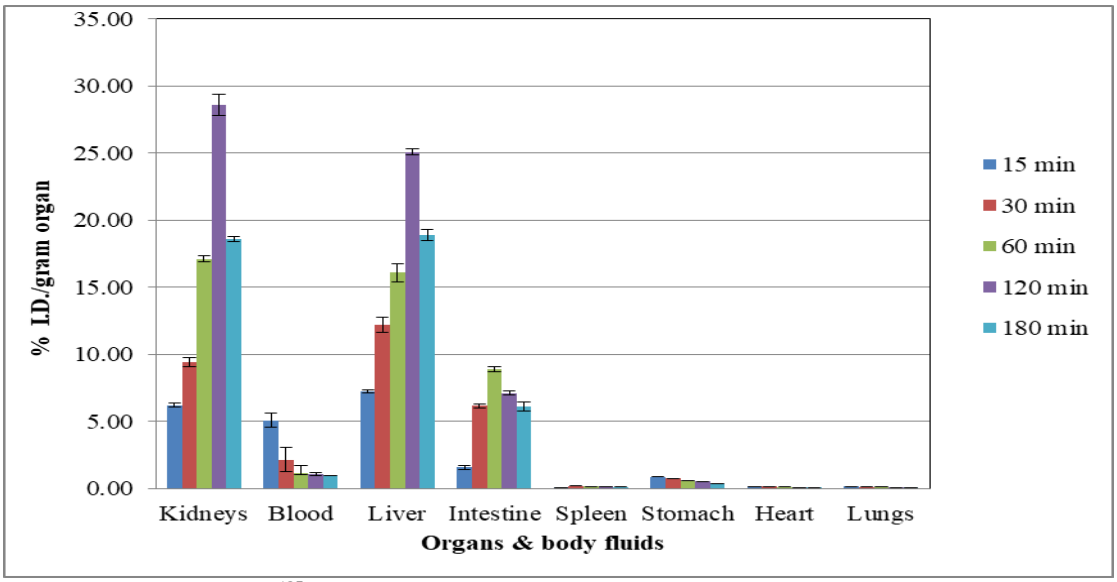

Figure (9): Biodistribution of [^{125}I] PA in mice organs.
Bars represent SE of three replicates.
Results showed no significant difference $P > 0.05$.

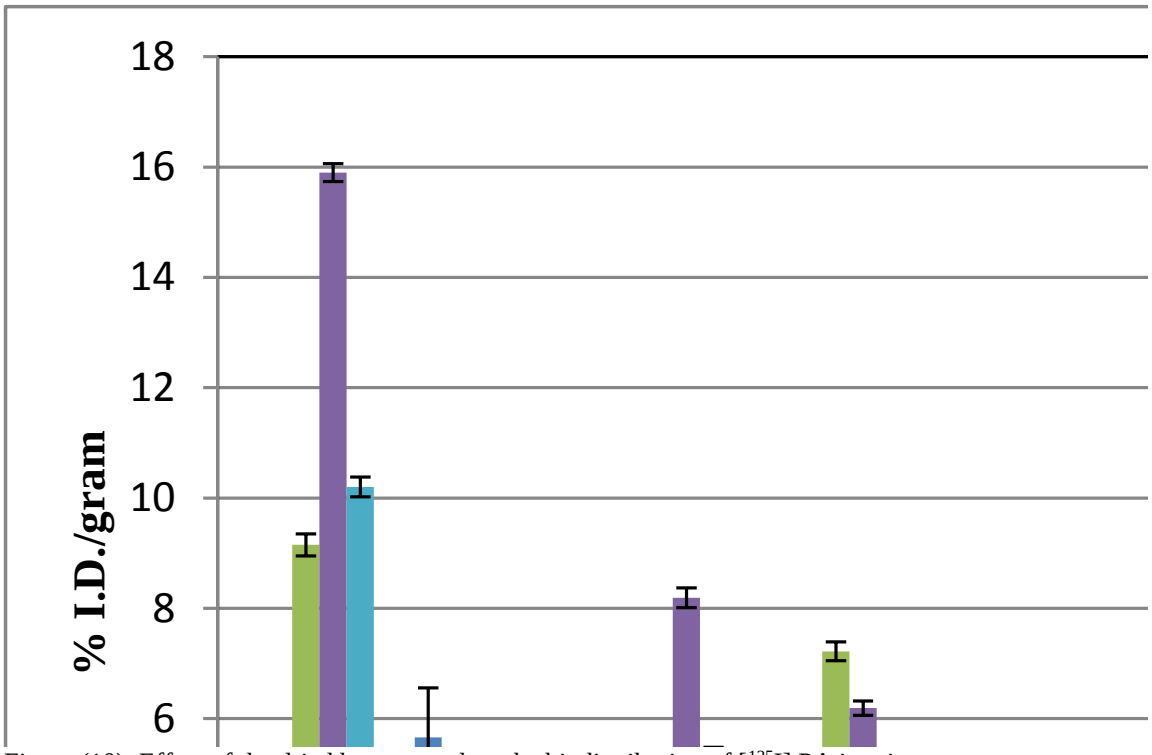

Figure (10): Effect of the dried banana peel on the biodistribution of [^{125}I] PA in mice organs.
Bars represent SE of three replicates.
Results showed no significant difference $P > 0.05$.

4. Conclusions

The results of this study revealed that [^{125}I] PA was distributed in several mice organs after 15 min. of injection and up to 120 min. post-injection, whereas in the second group of mice which received both [^{125}I] PA, and dried banana peels, [^{125}I] PA accumulated at a lower percentage in all organs. These results offer a possible approach to the use of agricultural waste to reduce the intestinal absorption of [^{125}I] PA from the animal diet.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

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