Data Article

Data on the *in vitro* and *in vivo* anti-tumor effects of itraconazole, paclitaxel, and the two in combination in HT-29 and YM-1 cancer cell line and HT-29 colon cancer xenograft models

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**ABSTRACT**

Colon cancer is one of the fatal cancers in the world that metastatic potential and resistance to chemotherapy drugs are outstanding causes of cancer-induced mortality [1–4]. We have investigated *in vitro* and *in vivo* anti-cancer effect of itraconazole and paclitaxel alone and their anti-cancer synergistic effect through MTT assay in YM-1 and HT-29 cell lines and in HT-29 tumor-bearing nude mice. Histopathological experiment was done for further assessment. Also, we evaluated the inhibitory effect of itraconazole on P-gp using specific *in vivo* biodistribution through \(^{99m}\)Tc-MIBI uptake. \(^{99m}\)Tc-MIBI, a myocardial perfusion imaging agent, is a useful radiotracer in diagnosis of some tumors and the liver and tumor accumulation of \(^{99m}\)Tc-MIBI is changed by P-gp regulators [5–8]. The data presented in this article are related to the research paper entitled "Itraconazole synergistically increases therapeutic effect of paclitaxel and \(^{99m}\)Tc-MIBI accumulation, as a probe of P-gp activity, in HT-29 tumor-

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bearing nude mice”. We hope our preliminary data to be helpful to design the chemotherapy regimen schedule with Itraconazole and Paclitaxel.
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### Specifications Table

| Subject                  | Health and medical sciences |
|--------------------------|-----------------------------|
| Specific subject area    | *In vitro* and *in vivo* anti-cancer effect of Itraconazole, Paclitaxel and the two in combination in YM-1 and HT-29 cell lines and HT-29 colon cancer xenograft models. |
| Type of data             | Table                       |
| How data were acquired   | ELISA reader (Biotech, USA), Gamma counter with a NaI (TI) detector ([Delshid, Tehran, Iran]) Excel 2016, Graf pad prism 8 |
| Data format              | Raw analyzed                |
| Parameters for data collection | Cytotoxic effects of paclitaxel, Itraconazole and concomitant treatment in Human colorectal cancer cell line (HT-29) and esophageal cancer cell line (YM-1) were evaluated by MTT test. cell viability % percentage was obtained through following equation: Absorption of Treated / Absorbed Control Cells) × 100%. *In vivo* anti-tumor efficacy of paclitaxel, Itraconazole and their synergistic effect was illustrate by measurement of tumor volumn by external vernier caliper during the 12 days' treatment period. P-gp medulatory effect of Itraconazole also assessed by biodistribution pattern of 99mTc-MIBI in HT-29 xenograft model, too. |
| Description of data collection | Human colorectal cancer cell line (HT-29) and esophageal cancer cell line (YM-1) were seeded in 96-well plates with 200 μl of RPMI and DMEM medium and incubated at 37°C for 24 h. Afret that, the medium was removed and cells were exposed to paclitaxel alone (0.05μM), Itraconazole alone (5μM), and the two in combination (Itraconazole + Paclitaxel) with the same concentrations (n=24). After 48 and 72 h incubation, 20 μl of MTT solution (5mg/ml) was added to the each well and incubated at 37°C for 4 hours. Then, the supernatant was removed and 100 μl of DMSO was added and absorbance of plates were read on an ELISA reader at a wavelength of 570 nm. For animal study, 1 × 10⁷ cell of HT-29 cell line were subcutaneously injected into the right hind legs of the Twenty-four female nude mice (3 to 5-week-old, 14-20 g), when tumors appeared, they divided to some extent uniformly based on tumor size into 4 following experimental groups. Control group, Itraconazole group (10 mg/Kg, i.p), Paclitaxel group (20 mg/Kg, i.p), Itraconazole (10 mg/Kg, i.p)+ Paclitaxel (20 mg/Kg, i.p) group. The daily tumor size measurement was started on 12th days after HT-29 cell transplantation and continued until the end of the treatment period. The treatment schedule was started on 14th day and drugs were injected intraperitoneally on 14th, 18th, 22th, and 26th days after HT-29 cells transplantation. Sestamibi kit (MIBI) was prepared according to the manufacturer's protocol and intravenously injected through the tail vein of nude mice 24 hours after final treatment day. 60 min after radiotracer injection, mice were sacrificed with a lethal dose of ketamine/xylazine (i.p.) and blood collected and other organs ranging from heart and liver to tumor were removed, weighed and counted by a gamma counter. |
| Data source location      | Institution: Mazandaran University of Medical Sciences City/Town/Region: Sari, Farahabad Country: Iran |

(continued on next page)
Value of the Data

• The presented data provided experimental evidence for the potential anticancer effect of Itraconazole and Paclitaxel and the two in combination in human colon cancer in both \textit{in vitro} and \textit{in vivo} xenograft model.
• The data provided valuable information of anti-tumor efficacy of Itraconazole, a commercially cheap and available antifungal drug, and paclitaxel, a commonly used drug for the treatment of ovarian, breast, head and neck and lung cancer, and their Synergistic anti-tumor effects in nude mice xenograft model of HT-29 cells. These data may give additional insight to oncologist to design new chemotherapy regimen with Itraconazole and Paclitaxel in colon cancer.
• The data provided valuable evidence for researchers to investigate the anti-cancer effect of Itraconazole and Paclitaxel and the two in combination using another similar cellular model.

1. Data Description

Recently we have reported \textit{that in vitro} and \textit{in vivo} anti-cancer effect of itraconazole, paclitaxel and the two in combination in YM-1 and HT-29 cell lines and HT-29 bearing nude mice [9]. Here, we present raw data regarding the anti-cancer effect of these drugs. Detail raw and analysis data on Cytotoxic effects summarizes in Table 1. Table 2 demonstrates the changes in tumor volume measurements during treatment period in itraconazole, paclitaxel and itraconazole+ paclitaxel-treated nude mice. Notable, the animal experiment was performed by following two treatment protocol: 1) itraconazole (20 mg/Kg), paclitaxel (20 mg/Kg), itraconazole (20 mg/Kg)+paclitaxel (20 mg/Kg). 2) itraconazole (10 mg/Kg), paclitaxel (20 mg/Kg), itraconazole (10 mg/Kg)+paclitaxel (20 mg/Kg). Itraconazole-induced liver toxicity at dose of 20 mg/Kg caused to lessen the dose of itraconazole to 10 mg/Kg. Fig. 1 shows the itraconazole-induced hepatotoxicity at dose of 20 mg/Kg. 99mTc-MIBI biodistribution raw data are presents in Table 3.

2. Experimental Design, Materials and Methods

2.1. MTT assay test

Human colorectal cancer cell line (HT-29) and Human ovarian cancer cell line (SKOV3) cultured in RPMI and DMEM medium supplemented with 10% fetal bovine serum and penicillin-streptomycin at 37°C and 5% CO2. Then these cells were seeded in 96-well plates and respectively incubated with 200 µl of RPMI and DMEM at 37°C for 24 h, again. After incubation, the medium was removed and cells were treated with paclitaxel (0.05µM), itraconazole (5µM), and combination treatment of itraconazole (5µM) + paclitaxel (0.05µM). It is notable that 10^4 cells were seeded in each well and the total dishes for each group were 24. After 48 and 72 h incubation, 20 µL of MTT solution (5mg/ml) was added to each well and incubated at 37°C for 4 hours, again. Then, the supernatant was removed and 100 µL of DMSO was added and mixed slowly. Finally, the absorbance of plates was read on an ELISA reader at a wavelength of 570 nm (Biotech, USA). The untreated cells (24 dishes) were used as a control group to compare
| Table 1 |
| --- |
| **The in vitro cytotoxicity of iraconazole alone, paclitaxel alone and iraconazole+ paclitaxel co-treatment in YM-1 and HT-29 cell lines.** |

| Cell | Control | ITCZ | PTX | ITCZ+PTX | Control | ITCZ | PTX | ITCZ+PTX |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **YM-1** | | | | | | | | |
| Mean | 100 | 97.2637 | 52.05222 | 30.08137 | 100 | 96.7485807 | 35.3640898 | 34.9849358 |
| SD | 25.24723 | 29.21545 | 27.07228 | 14.77387 | 16.73059 | 22.0196777 | 19.9338019 | 6.021506133 |
| **HT-29** | 107.413 | 111.8305 | 100.2057 | 10.79219 | 77.71545 | 95.6654768 | 12.2386537 | 9.790922998 |
| 95.3236 | 83.93057 | 83.69847 | 16.73969 | 121.2647 | 63.84497705 | 18.35798062 | 17.84803672 |
| 92.76581 | 74.86363 | 62.54136 | 16.5072 | 99.64304 | 89.75012749 | 47.01682815 | 37.53187149 |
| 83.93097 | 95.55575 | 75.65895 | 54.40401 | 105.7624 | 145.3304133 | 31.6165228 | 12.952755272 |
| 89.74336 | 85.32594 | 62.07637 | 77.18859 | 71.49414 | 83.7237893 | 43.24324324 | 3.36053034 |
| 94.62577 | 73.23616 | 100.6707 | 53.93901 | 102.8047 | 108.5160632 | 70.47424783 | 11.72789084 |
| 135.545 | 157.3996 | 62.77346 | 46.03914 | 94.44161 | 83.22284549 | 49.26058134 | 21.7236104 |
| 129.5001 | 95.09076 | 53.70652 | 22.55209 | 133.5033 | 82.1096869 | 77.51473744 | 11.28765964 |
| 79.74604 | 84.39596 | 23.01709 | 45.10418 | 99.43906 | 95.25752167 | 12.23865375 | 13.87047425 |
| 131.1276 | 88.34839 | 61.61131 | 41.38424 | 97.90332 | 100.6629291 | 11.32075472 | 14.07247345 |
| 109.273 | 92.53331 | 60.69881 | 37.19392 | 123.8144 | 73.0239673 | 46.30290668 | 23.59408047 |
| 71.84119 | 48.39512 | 109.505 | 30.67994 | 72.61601 | 78.02141764 | 46.24096352 | 14.07458141 |
| 110.9005 | 99.7406 | 46.73165 | 29.24946 | 83.12086 | 127.485795 | 44.46708162 | 10.54044447 |
| 70.67871 | 136.7075 | 30.68944 | 28.82897 | 97.2973 | 107.5981642 | 11.28765964 | 17.84083672 |
| 76.4911 | 75.09613 | 24.41205 | 25.80703 | 83.01883 | 130.9535951 | 50.28046915 | 13.78564857 |
| 56.03148 | 73.46866 | 48.84214 | 55.57453 | 124.4263 | 111.779042 | 45.58898552 | 1.21431525 |
| 108.808 | 100.9005 | 28.82897 | 24.64445 | 107.0831 | 114.4420750 | 9.28911798 | 10.30086969 |
| 122.0603 | 94.85827 | 34.17688 | 23.48027 | 97.39929 | 90.15808261 | 11.2876594 | 12.13666497 |
| 47.66163 | 96.02075 | 29.75465 | 23.15919 | 95.2573 | 114.7373789 | 37.3584906 | 10.60633235 |
| 73.70115 | 110.203 | 3.719322 | 22.0871 | 89.44416 | 76.28768036 | 31.20875472 | 10.54044471 |
| 119.9678 | 28.82897 | 22.31599 | 21.15711 | 122.3865 | 66.19071902 | 46.30290668 | 14.22623131 |
| 139.9624 | 114.6204 | 38.82679 | 20.92462 | 99.84702 | 76.18519598 | 46.30439632 | 12.633666497 |
| 135.08 | 88.34389 | 22.55209 | 20.69212 | 97.2973 | 120.83670588 | 18.35799062 | 14.07458141 |
| 117.1779 | 152.5172 | 79.04856 | 20.69212 | 93.82968 | 80.06169327 | 46.30290668 | 12.633666497 |
| 95.78825 | 142.7524 | 61.84387 | 19.76214 | 77.71545 | 95.6654768 | 12.23865375 | 17.90922998 |
| 104.8556 | 113.9229 | 39.05929 | 18.13467 | 121.2647 | 63.84497705 | 18.35799062 | 17.90843672 |
| **Mean** | 101 | 97.2637 | 52.05222 | 30.08137 | 100 | 96.7485807 | 35.3640898 | 14.8948358 |
| **SD** | 25.24723 | 29.21545 | 27.07228 | 14.77387 | 16.73059 | 22.0196777 | 19.9338019 | 6.021506133 |

The survival % = (absorption of treated / absorbed Control Cells) × 100%.
Table 2
Tumor volume changes in itraconazole, paclitaxel and the two in combination treatment groups in the HT-29 tumor-bearing nude mice.

| Days | ITCZ (10 mg/Kg), PTX (20 mg/Kg) | ITCZ (20 mg/Kg), PTX (20 mg/Kg) |
|------|-------------------------------|---------------------------------|
|      | mice 1 | mice 2 | mice 3 | mice 4 | Mean | SD   | mice 1 | mice 2 | mice 3 | mice 4 | Mean | SD   |
| 12   | 7.32667 | 6.28  | 23.81167 | 32.45504 | 17.46834 | 12.81759 | 41.448 | 73.476 | 57.776 | 57.56667 | 16.01503 |
| 13   | 10.46667 | 9.42  | 50.24   | 56.52   | 31.66167 | 25.2125 | 212.421 | 367.38 | 301.44 | 293.747 | 77.76541 |
| 14   | 15.7    | 25.12 | 94.2    | 161.1867| 74.05167 | 67.81762 | 233.4716 | 353.4824 | 320.28 | 302.4113 | 61.96867 |
| 15   | 51.025  | 37.68 | 234.4533| 314     | 159.2896 | 136.7419 | 273.9367 | 519.042 | 512.8667 | 435.2818 | 139.763 |
| 16   | 53.1    | 45.2  | 345.45  | 472.1   | 228.9625 | 213.9949 | 433.3828 | 574.62 | 586.1333 | 531.3787 | 85.06197 |
| 17   | 55.27708| 58.61333| 444.8333| 518.1   | 269.2059 | 248.9197 | 565.2   | 746.0483| 653.12 | 654.7894 | 90.43571 |
| 18   | 64.10833| 87.9032 | 548.5   | 636.1117| 334.4192 | 300.6149 | 653.12  | 775.58  | 707.5467 | 712.0822 | 61.35586 |
| 19   | 100.48  | 175.84 | 765.375 | 988.4383| 507.0446 | 436.4964 | 583.569 | 817.4456| 774.8997 | 725.3048 | 124.5765 |
| 20   | 139.2067| 233.5375| 769.3   | 1260.187| 600.5577 | 519.9785 | 521.4619| 803.1492| 820.482 | 715.031 | 167.8597 |
| 21   | 139.2067| 301.44 | 807.765 | 1289.493| 634.4763 | 521.3063 | 582.1649| 895.2564| 912.798 | 796.7398 | 186.0342 |
| 22   | 139.2067| 301.44 | 1036.2  | 1549.59 | 756.6092 | 657.1034 | 605.2973| 1148.063| 1033.256 | 928.872 | 286.043 |
| 23   | 167.4667| 334.9333| 1408.29 | 1604.933| 878.9045 | 732.4421 | -      | -      | -      | -      | -      |
| 24   | 244.6583| 853.24 | 1387.88 | 1921.68 | 1097.365 | 721.0438 | -      | -      | -      | -      | -      |
| 25   | 287.8333| 875.0133| 1730.925| 2197.477| 1272.812 | 855.0437 | -      | -      | -      | -      | -      |
| 26   | 366.3333| 1122.55| 2373.84 | 2197.477| 1515.05  | 944.6044 | -      | -      | -      | -      | -      |

ITCZ

| Days | mice 1 | mice 2 | mice 3 | mice 4 | Mean | SD   |
|------|-------|-------|-------|-------|------|-----|
| 12   | 15.56282 | 17.20174 | 25.39304 | 20.02319 | 19.5452 | 4.311878 |
| 13   | 38.48775 | 25.48406 | 35.04058 | 40.95652 | 34.99223 | 6.787222 |
| 14   | 61.23   | 40.95652 | 73.27147 | 65.35043 | 60.35967 | 13.93463 |
| 15   | 112.5804 | 51.19565 | 127.4203 | 145.6232 | 109.2049 | 40.96567 |
| 16   | 99.792  | 67.32609 | 167.2826 | 244.5822 | 144.7458 | 78.5068 |
| 17   | 98.29565| 85.08955 | 232.087 | 307.1739 | 180.6638 | 107.3412 |
| 18   | 102.3913| 112.1754 | 294.887 | 384.0565 | 223.3775 | 138.9663 |
| 19   | 113.8079| 196.5913 | 432.6033| 443.0659 | 296.5171 | 166.6971 |
| 20   | 152.0511| 213.9978 | 496.9391| 482.7174 | 336.4264 | 179.0237 |
| 21   | 170.8885| 281.5761 | 552.913 | 498.6807 | 375.9966 | 180.109 |
| 22   | 250.5401| 309.3924 | 611.6174| 536.1333 | 426.9208 | 174.1295 |
| 23   | 264.3061| 324.1254 | 611.6174| 846.0072 | 511.514 | 269.6496 |
| 24   | 364.422 | 418.6667 | 728.1159| 989.2812 | 625.1214 | 290.8653 |
| 25   | 470.1695| 450.5217 | 767.9348| 1290.952 | 744.8945 | 391.9349 |
| 26   | 585.7683| 691.7101 | 804.5681| 1783.725 | 966.4203 | 552.1512 |

(continued on next page)
| Days | mice 1 (mg/Kg) | mice 2 (mg/Kg) | mice 3 (mg/Kg) | mice 4 (mg/Kg) | Mean (mg/Kg) | SD (mg/Kg) |
|------|---------------|---------------|---------------|---------------|--------------|------------|
| 12   | 20.41         | 5.966         | 20.3472       | 27.475        | 18.54955     | 9.031467   |
| 13   | 49.455        | 9.42          | 26.16667      | 50.24         | 33.82042     | 19.73156   |
| 14   | 61.245        | 26.16667      | 54.95         | 102.05        | 61.05556     | 38.30833   |
| 15   | 70.92         | 44.22167      | 65.94         | 102.05        | 70.73722     | 29.21111   |
| 16   | 91.214        | 47.25         | 85.62         | 142.31        | 91.72667     | 47.82331   |
| 17   | 113.124       | 51.28667      | 102.5733      | 184.2133      | 112.69111    | 67.03843   |
| 18   | 131.587       | 65.94         | 102.5733      | 230.2667      | 132.92672    | 86.26591   |
| 19   | 151.625       | 65.94         | 150.72        | 240.7333      | 152.4644     | 87.40972   |
| 20   | 76.93         | 167.4667      | 251.2         | 165.1989      | 165.1989     | 87.15713   |
| 21   | 76.93         | 157           | 294.375       | 176.1017      | 176.1017     | 109.9738   |
| 22   | 76.93         | 244.6583      | 306.15        | 209.2461      | 209.2461     | 118.6422   |
| 23   | 76.93         | 336.765       | 353.25        | 255.6483      | 255.6483     | 154.9939   |
| 24   | 116.7688      | 586.1333      | 512.8667      | 405.2563      | 405.2563     | 252.509    |
| 25   | 117.2267      | 777.15        | 615.44        | 503.2722      | 503.2722     | 343.9635   |
| 26   | 146.5333      | 967.12        | 703.36        | 605.6711      | 605.6711     | 418.9248   |

| Days | mice 1 (mg/Kg) | mice 2 (mg/Kg) | mice 3 (mg/Kg) | mice 4 (mg/Kg) | Mean (mg/Kg) | SD (mg/Kg) |
|------|---------------|---------------|---------------|---------------|--------------|------------|
| 12   | 15.2473       | 8.007         | 21.2473       | 17.6867       | 15.64767     | 6.445289   |
| 13   | 28.65333      | 23.38646      | 28.26         | 37.68         | 29.77549     | 9.61085    |
| 14   | 78.9225       | 51.28667      | 71.435        | 119.0583      | 80.59333     | 34.80167   |
| 15   | 114.1         | 58.61333      | 117.75        | 164.85        | 113.7378     | 53.23186   |
| 16   | 147.3975      | 62.31         | 180.625       | 192.81        | 145.2483     | 72.08463   |
| 17   | 169.2083      | 69.60333      | 207.9596      | 226.865       | 168.1426     | 85.85948   |
| 18   | 192.5998      | 84.51833      | 240.21        | 253.555       | 192.7611     | 93.97817   |
| 19   | 204.8188      | 98.125        | 240.21        | 268.47        | 202.2683     | 91.29092   |
| 20   | 218.0813      | 104.6667      | 268.47        | 282.2          | 218.5789     | 98.90354   |
| 21   | 230.8758      | 104.6667      | 313.215       | 274.75        | 230.87722    | 110.9807   |
| 22   | 155.43        | 304.9725      | 311.3833      | 257.2619      | 257.2619     | 88.24729   |
| 23   | 172.7         | 366.3333      | 313.215       | 284.0828      | 284.0828     | 100.0499   |
| 24   | 207.24        | 387.79        | 366.3333      | 320.4544      | 284.0828     | 98.63179   |
| 25   | 219.8         | 397.7333      | 375.0338      | 330.8557      | 330.8557     | 96.84443   |
| 26   | 248.5833      | 492.195       | 471           | 403.9261      | 403.9261     | 134.9475   |
Table 3
The $^{99m}$Tc-MIBI accumulation in vital organs of HT-29 xenograft nude mice (n= 3 for each group).

| Control | Weight of organs | Cpm | %CPM/ID *W (except intestine: %CPM/ID) | Mean | SD |
|---------|------------------|-----|---------------------------------------|------|----|
|         |                  | 2539675 | 2528779 | 2012263 |      |    |
| blood   | 0.51 0.407 0.681 | 1727 | 3071 | 5879 | 0.133335 | 0.298383 | 0.429014 | 0.2869 | 0.1482 |
| heart   | 0.13 0.228 0.133 | 58559 | 94246 | 33479 | 17.73667 | 16.34621 | 12.50939 | 15.5308 | 2.7074 |
| s.a.    | 0.136 0.179 0.114 | 13910 | 18707 | 10535 | 4.027263 | 4.13276 | 4.592454 | 4.2508 | 0.3005 |
| lung    | 0.239 0.16 0.142 | 14363 | 7247 | 8795 | 2.366296 | 1.791131 | 3.077958 | 2.4118 | 0.6446 |
| liver   | 0.724 0.866 0.832 | 189273 | 264911 | 174638 | 10.29371 | 12.09682 | 10.43111 | 10.9405 | 1.0037 |
| kidney  | 0.334 0.381 0.264 | 324483 | 374505 | 164583 | 38.25316 | 38.87064 | 30.98106 | 36.0350 | 4.3877 |
| spleen  | 0.149 0.13 0.089 | 11843 | 9683 | 7379 | 3.129661 | 2.945477 | 4.120242 | 3.3985 | 0.6318 |
| stomach | 0.277 0.271 0.241 | 21703 | 12567 | 17759 | 3.085047 | 1.833798 | 3.661986 | 2.8603 | 0.9346 |
| muscle  | 0.156 0.158 0.192 | 7019 | 8047 | 8019 | 1.771628 | 2.01403 | 2.075555 | 1.9537 | 0.1607 |
| bone    | 0.042 0.111 0.332 | 1859 | 4175 | 7673 | 1.742817 | 1.487382 | 1.14853 | 1.4596 | 0.2981 |
| tumor   | 2.406 0.381 2.809 | 118999 | 13613 | 131259 | 1.947464 | 1.412921 | 2.322162 | 1.8942 | 0.4570 |
| intestine | - - - | 537311 | 472979 | 406355 | 21.15668 | 18.70385 | 20.19393 | 20.0182 | 1.2358 |
| tail    | - - - | 54563 | 71459 | 482891 | - | - | - | - | - |
| syringe | - - - | 177479 | 171479 | 276563 | - | - | - | - | - |
| ITCZ    | Injection Dose= (Total-Tail-Syringe): | | | | 2093707 | 2589595 | 203781 |      |    |
|         |                  | 2539675 | 2528779 | 2012263 |      |    |
| blood   | 0.184 0.238 0.424 | 4131 | 4399 | 3047 | 1.072313 | 0.713748 | 0.352649 | 0.7129 | 0.3598 |
| heart   | 0.18 0.152 0.083 | 36123 | 41303 | 24602 | 9.585071 | 10.49316 | 14.54549 | 11.5412 | 2.6411 |
| s.a.    | 0.104 0.12 0.103 | 8583 | 9455 | 10007 | 3.941756 | 3.042625 | 4.767632 | 3.9173 | 0.8628 |
| lung    | 0.163 0.115 0.119 | 7403 | 4895 | 4775 | 2.169223 | 1.643701 | 1.969076 | 1.9273 | 0.2652 |
| liver   | 0.939 1.1 1.074 | 252611 | 413483 | 272447 | 12.84904 | 14.51554 | 12.44841 | 13.2710 | 1.0963 |
| kidney  | 0.293 0.352 0.217 | 164866 | 274260 | 100991 | 26.87494 | 30.08762 | 22.83805 | 26.8002 | 3.6326 |
| spleen  | 0.129 0.105 0.073 | 10463 | 6383 | 5267 | 3.873919 | 2.347489 | 3.504597 | 3.2540 | 0.8026 |
| stomach | 0.302 0.384 0.189 | 18119 | 24887 | 9395 | 2.865572 | 2.502704 | 2.439332 | 2.6025 | 0.2300 |
| muscle  | 0.167 0.272 0.085 | 8191 | 14339 | 3203 | 2.342634 | 2.03572 | 1.849158 | 2.0758 | 0.2492 |
| bone    | 0.216 0.105 0.193 | 7015 | 6035 | 8491 | 1.55165 | 2.219505 | 2.158925 | 1.9765 | 0.3696 |
| tumor   | 4.619 2.975 1.567 | 263219 | 170831 | 67475 | 2.721782 | 2.217419 | 2.113051 | 2.3508 | 0.3255 |
| intestine | - - - | 546199 | 607319 | 479303 | 26.08765 | 23.45227 | 23.52048 | 24.3535 | 1.5022 |
| tail    | - - - | 497315 | 39551 | 608699 | - | - | - | - | - |
| syringe | - - - | 180695 | 142571 | 125207 | - | - | - | - | - |

(continued on next page)
| Injection Dose= (Total-Tail-Syringe) | PTX | ITCZ+PTX |
|--------------------------------------|-----|----------|
| **Blood**                           |     |          |
| M. Hosseinimehr et al. (2021)        |     |          |
| **Dose**                             |     |          |
| **Injection**                        |     |          |
| **Effect**                           |     |          |
| **Response**                         |     |          |
| **Tumor**                            |     |          |
| **Kidney**                           |     |          |
| **Spleen**                           |     |          |
| **Liver**                            |     |          |
| **Stomach**                          |     |          |
| **Muscle**                           |     |          |
| **Bone**                             |     |          |
| **Intestine**                        |     |          |
| **Tail**                             |     |          |
| **Syringe**                          |     |          |
| **Heart**                            |     |          |
| **Liver**                            |     |          |
| **Spleen**                           |     |          |
| **Stomach**                          |     |          |
| **Muscle**                           |     |          |
| **Bone**                             |     |          |
| **Intestine**                        |     |          |
| **Tail**                             |     |          |
| **Syringe**                          |     |          |
| **Heart**                            |     |          |
| **Liver**                            |     |          |
| **Spleen**                           |     |          |
| **Stomach**                          |     |          |
| **Muscle**                           |     |          |
| **Bone**                             |     |          |
| **Intestine**                        |     |          |
| **Tail**                             |     |          |
| **Syringe**                          |     |          |
| **Heart**                            |     |          |
| **Liver**                            |     |          |
| **Spleen**                           |     |          |
| **Stomach**                          |     |          |
| **Muscle**                           |     |          |
| **Bone**                             |     |          |
| **Intestine**                        |     |          |
| **Tail**                             |     |          |
| **Syringe**                          |     |          |
| **Heart**                            |     |          |
| **Liver**                            |     |          |
| **Spleen**                           |     |          |
| **Stomach**                          |     |          |
| **Muscle**                           |     |          |
| **Bone**                             |     |          |
| **Intestine**                        |     |          |
| **Tail**                             |     |          |
| **Syringe**                          |     |          |
cell absorbance and survival. The cell survival percentage was calculated according to following equation: Live cell percentage (\%) = (absorption of treated / absorbed control cells) \times 100%.

2.2. Effects of itraconazole and paclitaxel and their co-administration on tumor growth in HT-29 xenograft nude mice

HT-29 cells (1 × 10^7) suspended in 100 μl fresh medium and subcutaneously administered into the right hind legs of the mice using a grafting needle. The first tumor sizes were measured 12 days after transplantation by external vernier caliper and to assess the anti-tumor effects of itraconazole, paclitaxel and their contaminant treatment, the tumor volumes daily recorded till the end of treatment period. The tumor volume was calculated according to the standard formula \( \text{volume} = \frac{\text{length} \times \text{width} \times \text{height} \times \pi}{6} \) [10]. Also, 12 days after transplantation the tumor-bearing nude mice were divided uniformly based on tumor volume into 4 experimental groups: The first treatment schedule was control group, itraconazole group (20 mg/Kg, i.p), paclitaxel group (20 mg/Kg, i.p), itraconazole (20 mg/Kg, i.p) + paclitaxel (20 mg/Kg, i.p) group. Itraconazole-induced liver toxicity at dose of 20 mg/Kg caused to decrease the dose of itraconazole to 10 mg/Kg. The second treatment schedule was control group, itraconazole (10 mg/Kg, i.p), paclitaxel (20 mg/Kg, i.p), itraconazole (10 mg/Kg, i.p) + paclitaxel (20 mg/Kg, i.p) group. The treatment protocol was started 14 days after HT-29 cells transplantation and drugs were injected intraperitoneally on 14th, 18th, 22th, and 26th days for a total of 12 days of treatment period. It is noted that itraconazole was injected 30 min before paclitaxel administration.

2.3. 99mTc-MIBI biodistribution pattern

24 hours after the final drug injection, 99mTc-MIBI (10MBq in 0.1 ml) was intravenously injected to through the tail vein and mice were killed with a lethal dose of ketamine/xylazine (i.p) at 60 min after 99mTc-MIBI injection. Then blood was collected and necessary organs such as heart, lung, liver, spleen, salivary gland, stomach, kidney, muscle, bone, and tumor were removed, weighed and their radioactivity was counted by a gamma counter. 99mTc-MIBI uptake values were calculated as a percentage of injected doses per gram of tissue (%ID/g) except for intestine (%ID).

2.4. Statistical analysis

Data were analyzed using Excel and Graph Pad Prism 6 software (Graph Pad, La Jolla, CA, USA) and statistical analysis was done by ANOVA and independent-samples T-test.
Ethics Statement

The authors confirm that all experiments comply with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Also, ethical aspect of the research such as animal keeping and the minimum number of nude mice that needed for valid statistical analysis approved by the Research Ethics Guidelines of Mazandaran University of Medical Sciences.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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