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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 99mTc-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.

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 Figure
How data were acquired	ELISA reader (Biotech, USA), Gamma counter with a NaI (Tl) 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) lines were evaluated by MTT test. cell viability % percentage was obtained through following equation: Absorption of Treated / Absorbed Control Cells) $\times$ 100%. *In vivo* anti-tumor efficacy of paclitaxel, itraconazole and their synegestic effect was illustrate by measurment of tumor volum 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, 1×10^7 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 measurment was started on 12th days after HT-29 cell transplantation and cotinued untill the end of the treatment period.The treatment schedule was started on 14 th day and drugs were injected intraperitoneally on 14th, 18th, 22th, and 26^{th} 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 nud 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*)

Data accessibility	Data is with this article.
Related research article	Mahdi Ghadi[a], Seyed Jalal Hosseinimehr[a], Fereshteh Talebpour Amiri[b], Alireza Mardanshahi[c], Zohreh Noaparast[a*], Itraconazole synergistically increases therapeutic effect of paclitaxel and ^{99m}Tc-MIBI accumulationin in HT-29 tumor-bearing nude mice, European Journal of Pharmacology, https://doi.org/10.1016/j.ejphar.2021.173892

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 *in vitro* and *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 *that 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 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. ^{99m}Tc-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* cell cytotoxicity of itraconazole alone, paclitaxel alone and itraconazole+ paclitaxel co-treatment in YM-1 and HT-29 cell lines.

Cell survival %= (absorption of treated / absorbed Control Cells) × 100%.								
Cell	48 h				72 h			
	Control	ITCZ	PTX	ITCZ+PTX	Control	ITCZ	PTX	ITCZ+PTX
HT-29	107.413	111.8305	100.2057	16.97219	77.71545	95.6654768	12.23865375	9.790922998
	95.32326	83.93097	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	57.65895	54.40401	105.7624	145.3340133	31.61652218	12.95257522
	89.74336	85.32594	62.07637	77.18859	71.49414	83.73278939	43.24324324	13.36053034
	94.62577	73.23616	100.6707	53.93901	102.8047	108.5160632	70.47424783	11.72870984
	135.545	157.3996	62.77385	46.03416	94.44161	83.22284549	49.26058134	21.7236104
	129.5001	95.09076	53.70652	22.55209	133.5033	82.10096889	77.51147374	11.21876594
	79.74604	84.39596	23.01708	45.10418	99.43906	95.25752167	12.23865375	13.87047425
	131.1276	88.34839	61.61137	41.38424	97.09332	100.6629271	11.32075472	11.4227435
	109.273	92.53331	65.09881	37.19932	123.8144	73.02396736	46.30290668	23.55940847
	71.84119	48.35912	109.5055	30.68944	72.61601	78.02141764	48.24069352	14.07445181
	110.9005	99.74068	46.73165	29.29446	83.12086	127.4859765	44.46710862	10.50484447
	70.67871	136.7075	30.68944	28.82947	97.2973	107.5981642	11.21876594	17.84803672
	76.4911	75.09613	24.41205	25.80703	83.01887	130.9535951	50.28046915	13.76848547
	56.03148	73.46866	48.82411	25.57453	124.4263	111.7797042	45.58898521	17.13411525
	108.808	110.9005	28.82947	24.64455	117.0831	120.4487506	9.89291178	10.3008669
	122.0603	94.85827	34.17688	23.48207	97.39929	90.15808261	11.21876594	12.13666497
	47.66163	96.02075	29.75946	22.31959	95.25752	114.7373789	37.73584906	10.60683325
	73.70115	110.203	3.719932	22.0871	89.44416	76.28760836	11.32075472	10.50484447
	119.9678	28.82947	22.31959	21.15711	122.3865	66.19071902	46.30290668	12.44263131
	139.9624	114.6204	38.82679	20.92462	99.84702	76.18561958	48.24069352	12.13666497
	135.08	88.34839	22.55209	20.69212	97.2973	120.8567058	18.35798062	14.07445181
	117.1779	152.5172	79.04856	20.69212	93.82968	80.06119327	46.30290668	12.13666497
	95.78825	142.7524	61.84387	19.76214	77.71545	95.6654768	12.23865375	9.790922998
	104.8556	113.9229	39.05929	18.13467	121.2647	63.84497705	18.35798062	17.84803672
Mean	100	97.2637	52.05222	30.08137	100	96.74485807	35.36460989	14.69488356
SD	25.24723	29.21545	27.07228	14.77387	16.73059	22.01967776	19.93380193	6.021501633
YM-1	104.6329	77.26301	60.15681	75.26728	79.15194	38.31959	38.0055	32.35179
	89.23735	74.41197	65.85887	62.43763	87.00432	37.37731	31.7236	31.7236
	103.4925	77.26301	73.27156	70.42053	99.25402	38.63369	38.94778	32.03769
	86.95652	89.80756	73.27156	66.99929	91.40165	34.55045	39.57597	35.17864
	74.69708	86.38632	85.2459	68.99501	97.68355	33.92226	34.86455	37.37731
	110.6201	88.38204	72.98646	62.43763	73.81233	38.0055	40.20416	37.6914
	91.80328	96.07983	68.1397	68.70991	112.7601	35.80683	37.06321	32.66588
	88.09694	90.94797	53.88453	51.88881	121.5548	39.89007	35.49274	31.7236
	98.64576	99.78617	72.70135	65.85887	84.49156	32.66588	37.06321	33.92226
	100.9266	78.40342	72.70135	57.87598	120.2984	31.4095	35.80683	33.29407
	82.96507	83.25018	82.67997	82.10976	92.34393	32.35179	35.80683	32.35179
	88.09694	82.96507	69.56522	59.3015	83.23518	32.97998	37.37731	31.7236
	101.7819	84.10549	75.55239	58.73129	83.23518	34.86455	39.89007	32.03769
	103.7776	93.2288	82.67997	59.01639	78.20966	32.35179	35.80683	33.60817
	77.26301	78.68852	84.39059	51.0335	116.2152	32.97998	36.12093	34.55045
	110.6201	88.09694	67.28439	51.60371	116.2152	33.29407	40.20416	33.60817
	129.1518	93.5139	63.86315	59.3015	76.01099	38.0055	40.51826	40.20416
	110.9052	75.83749	76.9779	62.43763	129.0931	36.12093	44.60149	34.23636
	138.2751	91.51818	74.41197	76.9779	88.57479	36.43502	41.77464	33.29407
	107.484	92.08838	79.25873	57.87598	136.0031	43.9733	39.57597	32.97998
	94.08411	90.66287	82.10976	55.59515	100.1963	45.54378	37.06321	33.60817
	88.95225	85.2459	79.54383	57.87598	129.7212	31.7236	38.0055	43.9733
	98.64576	78.68852	80.39914	70.99073	88.57479	45.22968	51.51158	33.60817
	118.8881	93.5139	65.28867	74.41197	114.9588	34.23636	40.20416	32.35179
Mean	100	86.25564	73.42599	63.67308	100	36.27797	38.63369	34.17092
SD	15.27812	7.130796	8.074443	8.430188	19.06156	4.113065	3.83122	2.947897

Table 2
Tumor volume changes in itraconazole, paclitaxel and the two in combination treatment groups in the HT-29 tumor-bearing nude mice.

	ITCZ (10 mg/Kg), PTX (20 mg/Kg)						ITCZ (20 mg/Kg), PTX (20 mg/Kg)				
Days	mice 1	mice 2	mice 3	mice 4	Mean	SD	mice 1	mice 2	mice 3	Mean	SD
					Control						
12	7.326667	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	246.9197	565.2	746.0483	653.12	654.7894	90.43571
18	64.10833	87.92	549.5	636.1117	334.41	300.6149	653.12	775.58	707.5467	712.0822	61.35586
19	100.48	175.84	765.375	986.4833	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	521.3065	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	878.9056	732.4421	-	-	1285.83	1285.83	-
24	244.6583	835.24	1387.88	1921.68	1097.365	721.0438	-	-	1603.363	1603.363	-
25	287.8333	875.0133	1730.925	2197.477	1272.812	855.0437	-	-	1584.209	1584.209	-
26	366.3333	1122.55	2373.84	2197.477	1515.05	944.6044	-	-	1738.898	1738.898	-
					ITCZ						
12	15.56282	17.20174	25.39304	20.02319	19.5452	4.311878	24.2	82.68667	30.2	45.69556	32.17541
13	38.48775	25.48406	35.04058	40.95652	34.99223	6.787222	24.07333	83.73333	32.23733	46.68133	32.34657
14	61.23	40.95652	73.72174	65.53043	60.35967	13.93463	24.59667	178.7707	54.0708	85.81271	81.84172
15	112.5804	51.19565	127.4203	145.6232	109.2049	40.96567	26.16667	252.0373	69.0643	115.7561	119.9562
16	99.792	67.32609	167.2826	244.5826	144.7458	78.5068	31.92333	256.538	93.0696	127.177	116.1267
17	98.29565	85.09855	232.087	307.1739	180.6638	107.3412	28.3542	279.2088	106.132	137.8983	128.4088
18	102.3913	112.1754	294.887	384.0565	223.3775	138.9663	38.4336	320.28	122.46	160.3912	144.7012
19	113.8079	196.5913	432.6033	443.0659	296.5171	166.6971	36.4554	521.0935	124.9092	227.486	258.0892
20	152.0511	213.9978	496.9391	482.7174	336.4264	179.0237	-	546.3077	126.1338	224.1472	286.0384
21	170.8885	281.5761	552.913	498.6087	375.9966	180.109	-	599.0283	163.414	254.1474	309.65
22	250.5401	309.3924	611.6174	536.1333	426.9208	174.1295	-	664.738	200.2818	432.5099	328.4202
23	264.3061	324.1254	611.6174	846.0072	511.514	269.6496	-	678.24	235.5	456.87	313.0645
24	364.422	418.6667	728.1159	989.2812	625.1214	290.8653	-	819.54	317.9878	568.7639	354.651
25	470.1695	450.5217	767.9348	1290.952	744.8945	391.9349	-	836.81	328.9307	582.8704	359.1249
26	585.6783	691.7101	804.5681	1783.725	966.4203	552.1512	-	881.712	-	881.712	-

(*continued on next page*)

Table 2 (*continued*)

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	Mean	SD
						PTX					
12	20.41	5.966	20.3472	27.475	18.54955	9.031467	111.5385	28.888	21.98	54.13549	49.83228
13	49.455	9.42	26.16667	50.24	33.82042	19.73156	140.8974	33.45147	46.158	73.5023	58.71066
14	61.245	26.16667	54.95	102.05	61.05556	38.30833	163.8436	27.004	85.9732	92.2736	68.63701
15	70.92	44.22167	65.94	102.05	70.73722	29.21111	163.8436	24.88973	81.3888	90.04071	69.87979
16	91.214	47.25	85.62	142.31	91.72667	47.82331	163.8436	21.8858	81.3888	89.0394	71.28746
17	113.124	51.28667	102.5733	184.2133	112.6911	67.03843	252.4882	20.93333	79.1437	117.5217	120.4536
18	131.587	65.94	102.5733	230.2667	132.9267	86.26591	270.5231	20.93333	79.1437	123.5334	130.5817
19	151.625	65.94	150.72	240.7333	152.4644	87.40972	337.3085	22.4196	73.86745	144.5318	168.9196
20	-	76.93	167.4667	251.2	165.1989	87.15713	337.3085	20.93333	66.67267	141.6382	170.9917
21	-	76.93	157	294.375	176.1017	109.9738	346.9458	23.40347	66.67267	145.674	175.644
22	-	76.93	244.6583	306.15	209.2461	118.6422	388.1724	24.963	55.27708	156.1375	201.519
23	-	76.93	336.765	353.25	255.6483	154.9939	307.72	22.8592	47.77353	126.1176	157.7649
24	-	116.7688	586.1333	512.8667	405.2563	252.509	256.538	21.77067	41.5422	106.617	130.2112
25	-	117.2267	777.15	615.44	503.2722	343.9635	233.5806	17.79333	36.43447	95.93612	119.5674
26	-	146.5333	967.12	703.36	605.6711	418.9248	141.7026	18.4632	36.43447	65.53341	66.57362
						ITCZ+PTX					
12	15.2473	8.007	21.24733	17.68867	15.64767	6.445289	70.65	29.30667	51.28667	50.41444	20.68546
13	28.65333	23.38646	28.26	37.68	29.77549	9.610885	444.8333	29.30667	71.435	181.8583	228.7151
14	78.9225	51.28667	71.435	119.0583	80.59333	34.80167	316.512	39.564	76.5375	144.2045	150.3634
15	114.1	58.61333	117.75	164.85	113.7378	53.23186	359.4672	25.95733	73.476	152.9668	180.4059
16	147.3975	62.31	180.625	192.81	145.2483	72.08463	349.482	25.12	66.882	147.1613	176.4547
17	169.2083	69.60333	207.9596	226.865	168.1426	85.85948	326.089	31.1488	66.882	141.3733	160.9632
18	192.5998	84.51833	240.21	253.555	192.7611	93.97817	321.222	35.0424	61.544	139.2695	158.1317
19	204.8188	98.125	240.21	268.47	202.2683	91.29092	316.355	28.48608	48.56533	131.1355	160.7187
20	218.0813	104.6667	268.47	282.6	218.5789	98.90354	224.1332	17.0816	43.68525	94.96668	112.6496
21	230.8758	104.6667	313.215	274.75	230.8772	110.9807	178.2264	10.90208	33.74453	74.291	90.7324
22		155.43	304.9725	311.3833	257.2619	88.24729	178.2264	6.28	32.44667	72.31769	92.64807
23		172.7	366.3333	313.215	284.0828	100.0499	168.8064	4.291333	31.4	68.16591	88.20488
24		207.24	387.79	366.3333	320.4544	98.63179	158.256	4.291333	24.46374	62.33702	83.67837
25		219.8	397.7333	375.0338	330.8557	96.84443	148.6267	3.977333	22.50333	58.36911	78.71228
26		248.5833	492.195	471	403.9261	134.9475	125.8617	3.977333	21.98	50.60633	65.7917

Table 3
The ^{99m}Tc- MIBI accumulation in vital organs of HT-29 xenograft nude mice (n= 3 for each group).

	Injection Dose= (Total-Tail-Syring):						2539675	2528779	2012263		
Control	Weight of organs				Cpm			%CPM/ID*W (except intestine: %Cpm/ID)		Mean	SD
	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
	muacle	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-Syring)						2093707	2589595	2037811		
	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	26.6002	3.6326
	spleen	0.129	0.105	0.073	10463	6383	5267	3.873919	2.347489	3.540597	3.2540	0.8026
	stomach	0.302	0.384	0.189	18119	24887	9395	2.865572	2.502704	2.439332	2.6025	0.2300
	muacle	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	1.551165	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*)

Table 3 (*continued*)

	Injection Dose= (Total-Tail-Syring):						2539675	2528779	2012263		
PTX	Injection Dose= (Total-Tail-Syring)						2592595	2656027	2231159		
	blood	0.337	0.181	0.169	467	935	443	0.053451	0.194491	0.117486	0.1218	0.0706
	heart	0.15	0.129	0.142	21215	32003	19204	5.45528	9.340464	6.061397	6.9524	2.0902
	s.a	0.15	0.136	0.112	13023	9851	9404	3.348768	2.727149	3.763258	3.2797	0.5215
	lung	0.12	0.136	0.183	6071	6287	7080	1.951391	1.740492	1.73401	1.8086	0.1237
	liver	1.077	1.281	1.078	202795	181991	154280	7.262845	5.348946	6.414462	6.3421	0.9590
	kidney	0.256	0.32	0.283	156467	168191	116577	23.5748	19.78883	18.46273	20.6088	2.6528
	spleen	0.028	0.079	0.142	1859	4538	4638	2.560865	2.162743	1.463901	2.0625	0.5553
	stomach	0.16	0.276	0.262	10907	14195	11380	2.629363	1.936394	1.946751	2.1708	0.3971
	muacle	0.187	0.212	0.278	9895	11003	12171	2.040983	1.954082	1.962234	1.9858	0.0480
	bone	0.214	0.236	0.255	11063	15443	6267	1.993997	2.463696	1.101511	1.8531	0.6919
	tumor	0.182	0.9	1.015	4498	34811	28845	0.953264	1.456268	1.27372	1.2278	0.2546
	intestine	-	-	-	538115	644804	499538	20.75584	24.27701	22.38917	22.4740	1.7621
	tail	-	-	-	64511	16307	237918.3	-	-	-	-	-
	syringe	-	-	-	114611	99383	302639.7	-	-	-	-	-
ITCZ+PTX	Injection Dose= (Total-Tail-Syring)						2544835	2192971	2455027		
	blood	0.056	0.104	0.149	2040	3350	6563	1.431471	1.468854	1.794154	1.5648	0.1995
	heart	0.144	0.192	0.149	48755	55828	51504	13.30445	13.25922	14.07986	13.5478	0.4613
	s.a	0.131	0.164	0.097	20777	27709	11739	6.23235	7.704493	4.929502	6.2888	1.3884
	lung	0.113	0.14	0.189	10271	8399	16019	3.571697	2.735688	3.452369	3.2533	0.4522
	liver	0.84	1.139	1.025	257638	234910	257471	12.05233	9.404697	10.23171	10.5629	1.3545
	kidney	0.227	0.251	0.325	134713	122291	184255	23.31975	22.21712	23.09296	22.8766	0.5823
	spleen	0.087	0.154	0.168	7199	10271	11543	3.251571	3.041298	2.798679	3.0305	0.2266
	stomach	0.201	0.169	0.514	17135	12715	27095	3.349873	3.43081	2.147186	2.9760	0.7189
	muacle	0.348	0.09	0.523	15199	4743	24475	1.716232	2.403132	1.906183	2.0085	0.3547
	bone	0.37	0.155	1.094	14723	7223	55439	1.563633	2.124971	2.064152	1.9176	0.3080
	tumor	0.733	1.393	0.768	93935	115540	70409	5.035745	3.782233	3.734312	4.1841	0.7379
	intestine	-	-	-	550971	541315	794279	21.65056	24.68409	32.35316	26.2293	5.5161
	tail	-	-	-	43835	418199	46031	-	-	-	-	-
	syringe	-	-	-	183047	160547	270659	-	-	-	-	-

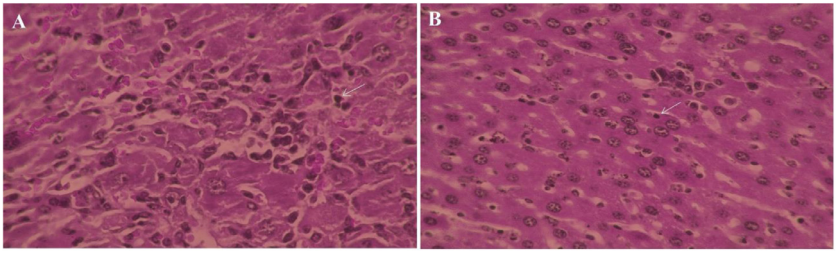

Fig. 1. Histopathologic findings of liver tissue A: Itraconazole (20 mg/Kg) group B: Itraconazole (20 mg/Kg) + Paclitaxel (20 mg/Kg) group. Hepatic cells showed increased inflammatory cells in itraconazole + paclitaxel groups but were only lower than those in itraconazole 20 mg / kg. H&E staining, Magnification: 40×, Scale bar: 100μm.

cell absorbance and survival. The cell survival percentage was calculated according to following equation: Live cell percentage (%) = (absorption of treated / absorbed control cells) × 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 administerd 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 (length × width × height × π)/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 14^{th}, 18^{th}, 22^{th}, and 26^{th} days for a total of 12 days of treatment period. It is noted that itraconazole was injected 30 min before paclitaxel administration.

2.3. ^{99m}Tc-MIBI biodistribution pattern

24 hours after the final drug injection, ^{99}mTc-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 ^{99}mTc-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. ^{99}mTc-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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