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DNA Extraction in Breast Cancer patients and study their drugs interactions

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Abstract

This study includes the extraction of DNA from patients with breast cancer; 52 samples were taken from patients with breast cancer with age ranged between (23- over 40) years. Extraction of DNA from WBC was done by using two methods, kit and manual method then a comparison between the two methods. An electrophoresis migration was carried out for 60 samples to ensure the purity of the extracted DNA. Study of the interaction between the drug and extracted DNA was caried out. The drug that was used is "capecitabine "which was prepared with different concentrations and mixed with DNA. The absorption of solutions was measured by using two apparatuses (IR, U.V) and the results of this part shows that, the drug interact with the double helical structure of DNA.

Keywords: Brest cancer, Capecitabine, DNA, Cancer and Drug interaction.

1. INTRODUCTION

Cancer regards as a second leading of death worldwide; in general, the prevalence of cancer has increased in the United States alone, and by 2014, about 1,665,540 people had cancer, and 585,720 of them died due to this disease.[1] Therefore, it is a common disease at the level of tissues, in addition, this variety makes it difficult, and to diagnose it is specifically.[2] Depending on the data, breast cancer and prostate cancer constitute a major portion of cancer in the men and women, respectively.[3] In the children, the most common cancer is blood cancer, and cancers related to the brain as well as lymph nodes, respectively.[4,5] Of all over the world, cancer regards as one of the most flourishing diseases. [6]

DNA is the second types of nucleic acid that contain the genetic materials specifying the biological development of all cellular forms of life and (many viruses). Therefore, it is often referred to as a molecular of life due to the responsible for the genetic propagation of all traits. [7,8,9]

A critical assumable of DNA is their ability to replicate itself or make copies of itself. Each strand of DNA double helix acted as configuration for duplicating the sequence of the base. This form is essential especially through cells divide because of each new cell perforce or needs to have an exact copy of DNA that existing into old cell. So that DNA is transferred to descendant cells because information stored in the sequence of strand through the" transcription process" gene is copied into RNA after that translate to the sequence e of amino acid to made protein. DNA repair of any damage to avoid changes. Since DNA is responsible for the transfer of genetic information, they are later to lead to the discovery of the central dogma of biology, which explains move of genetic materials through DNA to RNA and from RNA to protein. [10]

Recently research shows that they are a relationship between telomeres and ageing. [11,12] In the humans, length of telomere seems to decrease at rate of 24.8-27.7 base pairs per year. [11,12] Telomeres are repetitive the sequence of DNA at the ends of all human chromosomes [13] which is consist of thousands of the six- nucleotide sequence (CTTAGGG). [14] For a specific age group, telomere length shorter than average telomere length where it is associated with increased incidence of age-related diseases and decreased lifespan in humans. [15,16,17]

The sequence of telomeres has been shortening in every time that DNA replicated itself so that decrease of this enzyme leads to decrease of replication of DNA and graduation cause cells death[18] because DNA has stopped in the replication process, which is necessary to the continuous growth of cells. Despite cancer cell, contain an enzyme called telomerase, which acted as a protection chromosome from chromosomes fusion by adding back" telomeres"; which is regards as reverse transcriptase and it compensates for the loss of telomere enzyme in some of the cells. [19,20,21,22]

The aim of this study is divided into the two-parts, extraction of DNA by using two methods Kite method and Manual method and comparative the results to choose the better pathway for the extraction. Study the effect of the interaction of therapy that used for the treatment of Breast cancer with DNA by using two apparatus, which are (U.V-visible spectroscopy, IR) to fine the type of interaction between the drug and DNA.

2. MATERIALS AND METHODS

2.1. Samples

Fifty-two samples of blood with breast cancer were used in this study, and most of all samples were taken from the woman. The ageing patient is between (20- above 63) years. Those women visited the medical city, Kirkuk oncology hospitals during the period from 7th September 2017 till January 2018.

2.2. Extraction of DNA

2.2.1 Procedures of Kit method

The procedure of extraction of DNA from fresh as well as the frozen sample was done as follow:-

- The peripheral blood sample was collected in EDTA tube, 2 ml of which was transferred to a new 2 ml microtube.
- The sample was centrifuged for 10 min at 6000 rpm at 4°C
- Plasma was aspirated and transferred to the new microtube, without touching the leukocyte layer.

(Leukocyte layer, serum, plasma) After aspirated from each other is treated with some solution and reagents to extracted DNA from its. These steps are done as follow:-

Step 1: (blood sample preparation)

A. Transfer up to 200µl of (whole blood, serum, plasma) to the 1.5microcentrifuge tube.
B. Adjust the volume to 200 µl with "PBS", add 20 µl of proteinase k then mix by pipetting.
C. Incubate mixtures that contain (20 µl of proteinase +200µl of whole blood) at 60c°for five minutes.

Step 2: (cell lysis)

In this step added about 200µl of GSB buffer then mix by shaking vigorously. After addition of GSB buffer, incubate mix at 60 c° for 5 minutes with inverting the tube every two minutes. During incubation, transfer required volume of elution buffer (100µl for each sample) to a 1.5ml microcentrifuge tube and heat to 60 c° (for step 5, which is DNA elution).

Step 3: (DNA binding)

After the step, "cell lysis" added about 200 µl of absolute ethanol to the sample lysate and mixed immediately by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. In this step, place a GS column in a 2 ml collection tube and transfer all of the mixture (including any insoluble precipitate) to the GS column. Centrifuge membrane; increase the centrifuge time until it passes completely. Discard the 2ml collection tube containing the flow –through then transfer the GS column to a new 2ml collection tube. In this step, it is vital that lysate and ethanol be mixed thoroughly to yield a homogeneous solution.

Step 4: (wash)

1. Added about400 µl of washing buffer one 'W_1" to the GS column. After that centrifuge at 14-16,000□ g for 30 seconds then discard the flow-through. Place the column back in the 2ml collection tube.
2. Addition 600µl of wash buffer two"W_2" (make sure absolute ethanol was added) to the GS column. After addition, this amount of "W_2", centrifuge again at 14-16,000 □g for 30 seconds then discard the flow-through .place the GS column back in the 2mlcollection tube. Centrifuge again for 3 minutes at 3minutes at 14-16,000□g to dry the column matrix.

Step 5: (Elution)

- Transfer the dry GS column to a clean 1.5 ml centrifuge.
- Add 100µl of preheated elution buffer, buffer or water into the centre of the matrix. Let stand for at least 3 minutes to allow elution buffer, TE buffer or water to be absorbed entirely .centrifuge at 14-16,000□g for 30 seconds to Elute purified.

2.2.2. The procedure of Manuel method

Applicated on the whole blood, involve many steps which are done as follow:-

Step 1:

1. Addition 1ml of fresh or frozen (thawed in room temperature) whole blood into 2ml micro-tube and mixed in 1ml of ddH_2O.

2. A mixture that contains (1ml ddH_2O with 1ml whole blood)was mixed for 30 sec and then was centrifuged at 7000 rpm for 5min. The supernatant was discarded and repeated step one for 2times, and as well as in each time the pellet was break up and mixed by shaking and overtaxing.

Step 2: addition 1ml of buffer A which acted as (Red blood cell lysis buffer) to the pellet and again mixed with vortexing for the breakup and rinse.

Step 3: after addition of Buffer A, centrifuge mixture at 7000 rpm for 5 min and then the supernatant was removed. In this step, if the pellet has red blood cells, step two and step three should be repeated.

Step 4: in this step, added 1ml of buffer B and 10 ml of SDS 10% and the pellet was suspended with vortexing or shaking then tube incubated at 65c° for 15 min.

Step 5: micro tube chilled with ice for 3 min and 400µl of chloroform and 400 µl of saturated NaCl (5M) were added after that, gently was shaken for 30 sec and centrifuged at 5000 rpm for 10 min at 4c°.

Step 6:- about 1ml of the upper phases transferred into a new 2ml tube, an equal volume of absolute cold ethanol was added in Figure 19.

Step 7: shake the tube; DNA should appear in this step.

Step 8: tube again centrifuged at 13000rpm for 10 min at 4c°.

Step 9: the supernatant was discarded, and the pellet was washed with 1ml of chilled 70% ethanol and centrifuged at 10000 rpm for 10min.

Step 10: the supernatant was discarded, and the pellet was left at room temperature to be dried.

Step 11: 50- 100 µl of ddH_2O added to dissolve the pellet and DNA solution stored at -20c° until use.

2.3.

To evaluate the probability of DNA degradation, gel electrophoresis was carried out by loading 4ml of DNA that extracted by two methods on 1.5% agarose gel.

The procedure of preparation of gel agarose: -

The procedure of electro force: -

- Add 1.5 gm of gel agarose to conical flask.
- Added 10%of TAE buffer to a conical flask and mixed with gel agarose as well as shaking conical flask to solve, all gel in the buffer. After solving of gel, the conical flask is covered by a piece of aluminium, with exist some hole in the cover and place it in the Microwave apparatus for less than 2 minutes, with shaking of conical flask every 10 sec. Then we take the conical flask out of a Microwave, put it in one drop of 0.07% ethidium as indicted, and let it cool down for 5 minutes. After cooling, we put it in a steady phase like this figure. After that, connect a steady phase with an electric after that we mix 4µl with 2µl of loading colour and put it in holes that exist in a steady phase. 50 volts power the current and then increase to 150 volts during that period DNA is started to moving from a negative pole to appositive pole

3. RESULTS AND DISCUSSION

Before DNA extraction, C.B.C measurements and some parameters are made for only 30 sample that is using for DNA isolation by using the kit and manual method, which is shown in the table (1)

Table (1): This table shows the C.B.C measurement and some pare meters

number	Urea	Creatin	GOT	GPT	W.B.C	Hb	P.C.V	PLT
sample 1	29	0.58	23	16	8.5	10.4	30.3	216
sample 2	28	0.4	24	18	5.4	12.9	38.4	142
sample 3	19	0.5	25	32	7.7	12.1	34.2	245
sample 4	19	0.6	24	30	4.5	12.6	28.8	202
sample 5	35	0.8	18	14	3.2	9.6	24.9	129
sample 6	39	0.5	22	13	4.2	6.9	25.9	126
sample 7	24.6	0.6	20	14	5.3	11.2	24.7	132
sample 8	41.89	0.60	25	32	6.2	10.4	70.5	372
sample 9	38.55	0.80	51	31	3.1	8	23.2	129
sample10	32	0.7	25	35	6.5	3.95	10.8	184
sample11	27.5	0.77	32	50	4.0	11.3	3.82	334
sample12	19.3	0.5	21	13	3.0	10.9	32.2	217
sample13	62	1.0	19	9	6.4	10.4	30.2	195
sample14	34	0.7	20	8	3.5	8	24.2	151
sample15	44	0.3	26	16	4.9	11.2	33.4	225
sample16	32	0.55	25	17	9.8	10.9	31.8	236
sample17	38.2	0.67	23	15	4.8	11.3	32.2	173
sample18	34.4	0.6	28	29	6.6	13.5	30.5	172
sample19	51	0.4	52	81	2.6	12.5	36.1	166
sample20	18	0.2	13.1	14	4.9	9.6	28	119
sample21	22.8	0.5	32	24	3.2	13.0	36.6	207
sample22	29.5	0.52	31	22	6.6	12.1	34.4	421
sample23	23	0.8	56	35	8.5	11.8	35.4	344
sample24	35.6	0.7	25	17	8.0	15.7	46.6	189
sample25	22	0.8	22	11	4.9	9.6	28	119
sample26	19.3	0.5	21	13	3.0	10.9	32.2	217
sample27	39.6	0.61	30	42	7.0	11.9	36.4	220
sample28	22.3	0.76	20	26	6.1	10.6	30.6	211
sample29	18.6	0.6	14	15	5.1	12.6	35.7	195
sample30	13.8	0.43	28	13	6.8	12.6	35.3	178

The results of the analysis of many samples were normal and within limits allowed. This type of analysis is conduct for all patients with breast cancer to know the immunity of the patients and to diagnose the kidneys of the patients.

3.1.1 Results of extracting DNA from whole blood by the kit method.

After extraction of DNA from (whole blood), they measure the purity of DNA from RNA as well as protein. These points are very important which is regards as basically point for comparison between the results of the two methods, so that the result of Nano graph measurement for (52sample) is shown in the table (2), which is extracted DNA from whole blood, by using enzymatic method or kit method.

3.1.2. Discussion results of extracting DNA from whole blood by the manual method.

In the manual method, most of the reagent that is using to extraction DNA is organic so that this method is called "organic method ". The manual method is applicated on the 25 sample, and the results of the concentration of DNA in each sample are shown in the table (3).

Table (2): Results of Nano graph of a kit method for the 52 samples that are using to extraction DNA from whole blood (W.B.C part).

number	con.ng/µL	A260/280	A260/230	Age	Type	Year
sample 1	16.85	1.62	0.85	59	malicious 2	2017
sample 2	70.650	1.85	1.742	40	malicious 3	2015
sample 3	23.700	1.809	1.095	55	malicious 3	2016
sample4	53.800	1.282	0.305	35	malicious 2	2017
sample 5	16.900	1.444	0.441	45	malicious	2012
sample 6	19.950	1.523	0.489	55	malicious4	2014
sample 7	31.050	1,572	0.615	41	malicious	2015
sample 8	13.800	1.801	1.104	62	malicious 4	2009
sample 9	25.450	1.973	1.025	55	malicious	2013
sample10	9.4500	1.454	0.508	48	malicious	2016
sample11	23.750	1.801	1.115	50	malicious	2013
sample12	26.800	1.807	1.153	52	malicious	2017
sample13	13.250	1.636	0.810	33	malicious	2016
sample14	63.650	1.864	1.632	45	malicious	2017
sample15	89.250	1.883	1.462	34	malicious	2017
sample16	167.67	1.990	1.940	27	malicious	2017
sample17	37.750	1.230	0.277	37	malicious	2017
sample18	35.100	1.961	1.186	50	malicious	2010
sample19	14.00	1.805	0.833	70	malicious	2017
sample20	44.050	1.907	1.181	37	malicious	2017
sample21	35.150	1.905	1.244	42	malicious	2015
sample22	131.30	1.913	1.826	56	Benign	2014
sample23	28.300	2.00	1.076	37	malicious	2017
sample24	21.650	2.062	0.941	42	malicious	2014
sample25	42.250	1.947	1.154	56	malicious	2015
sample26	131.45	1.886	1.386	37	malicious	2016
sample27	35.900	1.899	1.630	29	Benign	2012
sample28	17.950	1.459	0.829	60	malicious	2016
sample29	30.950	1.636	0.807	51	malicious	2015
sample30	48.100	1.806	1.245	30	malicious	2015
sample31	37.400	1.630	0.819	45	malicious	2017
sample32	22.050	1.901	1.128	47	malicious	2017
sample33	33.900	1.837	1.980	30	Benign	2017
sample34	31.100	1.868	1.656	38	malicious	2014
sample35	41.050	1.918	2.089	28	Benign	2012
sample36	31.950	1.919	1.238	60	malicious	2017
sample37	69.150	1.808	0.763	54	malicious	2014
sample38	20.450	1.834	1.378	72	malicious	2017
sample39	84.200	1.877	1.378	54	malicious	2003
sample40	31.750	1.819	1.293	66	malicious	2014
sample41	17.150	1.836	1.117	50	malicious	2015
sample42	69.100	1.875	1.245	36	malicious	2016
sample43	8.3500	1.295	0.508	56	malicious	2016
sample44	50.950	1.912	1.915	74	malicious	2017
sample45	27.000	1.849	1.123	30	malicious	2017
sample46	21.600	1.636	0.891	45	malicious	2017
sample47	25.600	1.932	1.424	62	malicious	2017
sample48	25.650	1.865	0.908	80	malicious	2015
sample49	20.950	1.805	1.420	47	malicious	2017
sample50	8.8000	1.813	0.793	50	malicious	2017
sample51	29.600	1.810	1.355	50	malicious	2017
sample52	6.3500	1.283	0.718	45	malicious	2017

Figure (1) Shows the absorption and bands of all reading in the table (3).

Table (3): Shows the results of Nano graph of the concentration and absorption of the 25 sample, which extracts DNA from whole blood by using the manual method

number	con.ng/µL	A260/280	A260/230	Age	Type	Year
sample 1	19.850	1.757	1.582	59	malicious 2	2017
sample 2	41.550	1.818	0.923	40	malicious 3	2015
sample 3	21.700	1.964	3.945	55	malicious 3	2016
sample 4	31.000	1.956	2.279	35	malicious 2	2017
sample 5	43.250	1.935	2.703	45	malicious	2012
sample 6	27.150	1.953	1.692	55	malicious 4	2014
sample 7	2015	1.655	0.799	41	malicious 2	
sample 8	17.600	1.253	0.386	62	malicious 4	2009
sample 9	99.300	1.881	1.602	55	malicious	2013
sample10	33.50	1.800	1.705	48	malicious	2016
sample11	12.150	1.328	1.397	50	malicious	2013
sample12	729.85	1.834	1.660	52	malicious	2017
sample13	25.400	1.644	2.731	33	malicious	2016
sample14	54.550	1.809	1.777	45	malicious	2017
sample15	11.250	1.389	1.355	34	malicious	2017
sample16	11.700	1.410	3.250	27	malicious	2017
sample17	17.200	1.585	2.797	37	malicious	2017
sample18	1.2000	-0.774	-0.131	50	malicious	2010
sample19	9.2500	1.370	0.830	70	malicious	2017
sample20	328.35	1.840	1.573	37	malicious	2017
sample21	10.250	1.395	5.694	42	malicious	2015
sample22	14.100	1.492	1.306	56	benign	2014
sample23	1.7500	-1.591	0.897	37	malicious	2017
sample24	5.1000	1.146	1.522	42	malicious	2014
sample25	657.15	1.785	1.508	56	malicious	2015

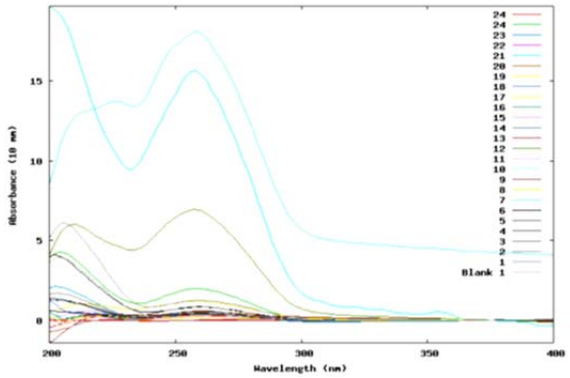


Figure (1): Shows the absorption and bands of all reading in the table (3) which represents the extraction of DNA from whole blood via the manual method.

There are several ways to measure the concentration as well as purity of DNA, which can determine the absence of the extract nuclear acids from (proteins, phenol, also RNA). To explain the results in the table (2) and table (3) that is using Nano graph measurement we should be now some basic point, which is: -

- The measurement method is depended on the fact that each material has a specific wavelength that absorbs the highest amount of the light. DNA is measured along a wavelength of 260 nm, which represents DNA absorption, while 280 is the higher absorption of protein as well as phenol, which is using to prepare some reagent that is using in some step of the kit method or manual method. In addition, 230 nm represents the highest absorption of RNA.
- The ratio 260/280 is utilised to express DNA purity from proteins and phenols through OD on two wavelengths 260nm, 280nm. If the ratio is between or equal to (1.8 – 2), DNA is pure, and if the percentage is lower, it is an indicator of DNA contamination by protein.

The ratio 260/230 is utilised to express the DNA purity of RNA by measuring OD on two wavelengths 260 nm and 230 nm. If the radio 260/230 is equal or between (1.5 -2) in these cases, DNA is pure, but, if this percentage is lower, it is an indicator of DNA contamination with RNA. Therefore, Table (2) and other tables explain as these points.

Dependence on these results we can compare between the two methods as this following table (4) base on the advantage disadvantage of each method.

Table (4): Compare between kit method and the manual method.

Manual Method	Kit Method
Cheep	Expensive
Need about 3000ml of blood; this amount is not constant "high yield."	Need about 200ml of blood and this is amount is constant "low yielded"
The tired method more time need (two days)	Short time needs "fast method."
Need to prepare reagents that a user in the assay	Not need to prepare Chemical material
Poisonous method	Safely assay

3.2. Agarose gel electrophoresis.

Electrophoresis technique is a process that is utilised for separation, as well as analysis of molecules especially macromolecules, which are involved (DNA, RNA, proteins). Figure (2); shows the electrophoresis of 45 samples that extraction from whole blood (W.B.C) part by the kit and manual method.

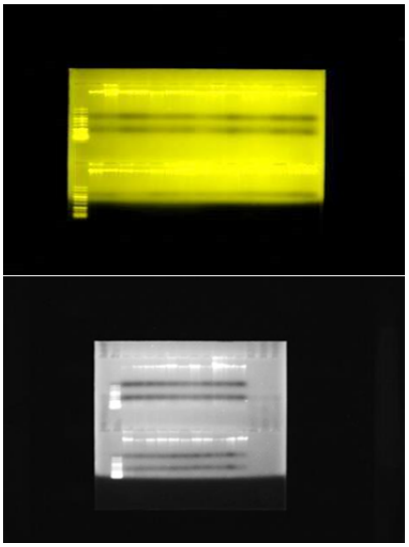


Figure (2): Shows the photo of the migrations of the 45 samples on 1% agarose gel; which are a return to the extraction of DNA from whole blood by using the kit method and manual method.

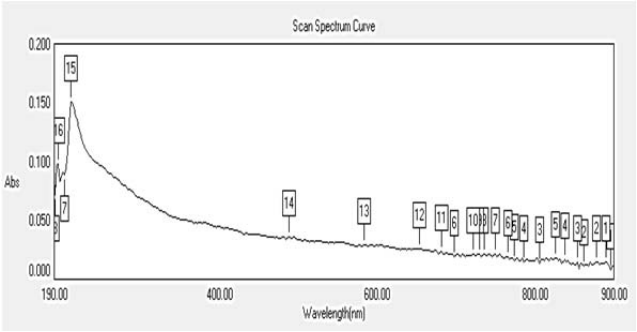
3.3. Interaction between DNA and drug.

To study the interaction of drugs with DNA utilises two types of DNA:

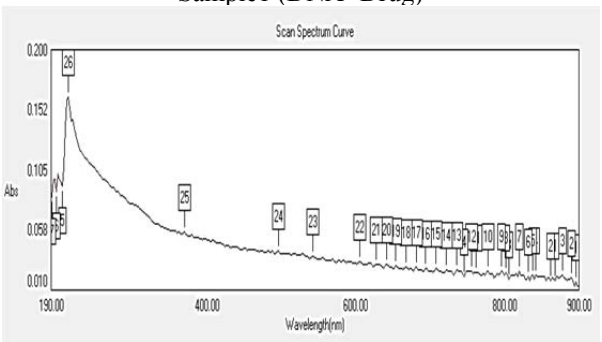
1. DNA that is extracted from some samples in the whole blood in the part one of this study.
2. DNA that extracted from calf tissue (solid DNA) and applicated the same procedure and methods to shows the types of interaction between DNA and drug that are using for this propose which is capecitabine.

In the Figure (3), shows the results of absorption (UV-visible spectroscopy) produce as a result of interaction of drug "capecitabine" for the five samples that are in addition of the drug, contain DNA which are extracted from whole blood by using kit method, and mixed with Tris –base. Also, figure (4), shows the results of the interaction of between DNA that is extracted from tissue "calf" and drugs via (UV-visible spectroscopy) in the same wavelength which is (190nm-9000nm).

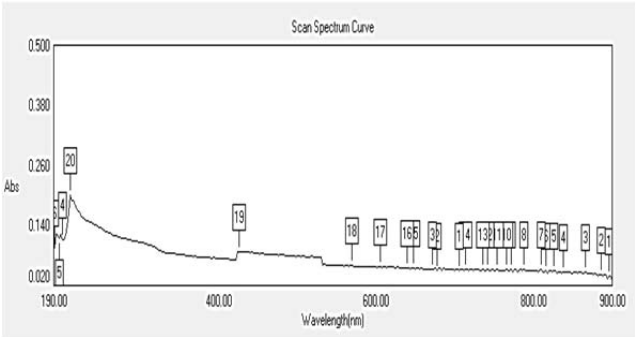
DNA Free



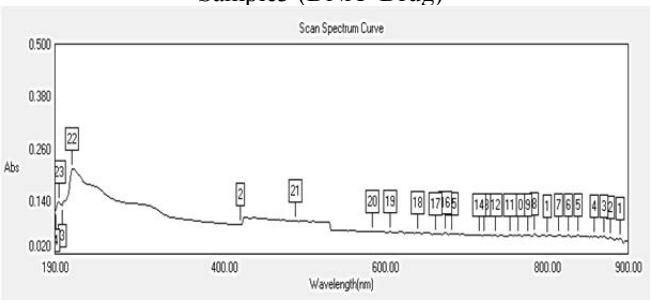
Sample1 (DNA+Drug)



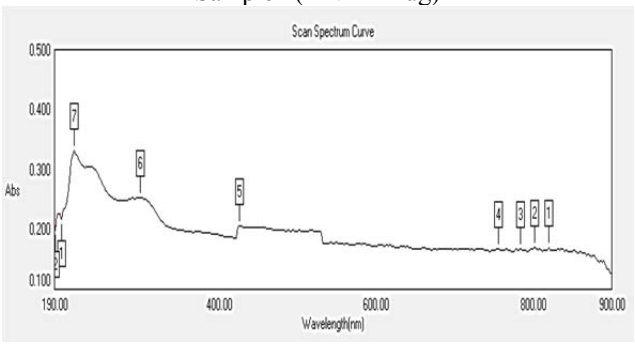
Sample2 (DNA+Drug)



Sample3 (DNA+Drug)



Sample 4(DNA+Drug)



Sample 5(DNA+Drug)

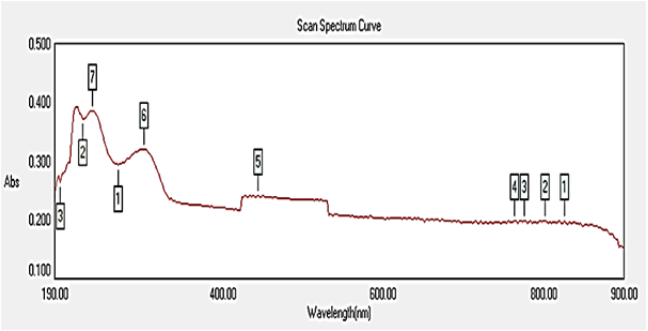


Figure (3): Shows the results of absorption (UV-visible spectroscopy) produce as a result of the interaction of drug "capecitabine" for the five samples that are also of the drug "different concentration", contain DNA which is extracted from whole blood by using the kit method.

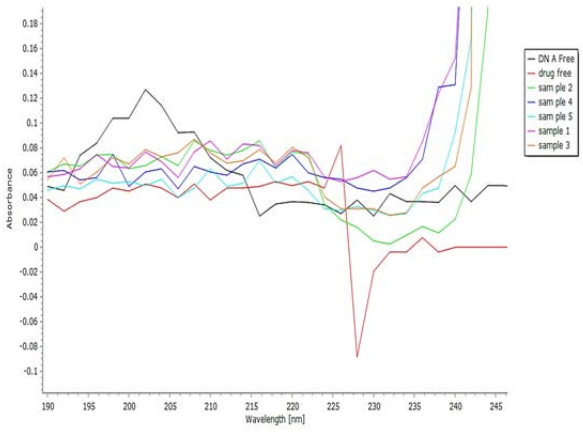


Figure (4): shows the results of the interaction of between DNA that is extracting from tissue "calf" and drugs via (UV-visible spectroscopy).

Depended on these results, we are observe increasing the intensity of the absorption for all samples that contain DNA and drugs in the different concentration compared with free DNA in the two results and the results of the free drug. Increasing in the absorption is proportional with increasing of the concentration of drugs in the solution (DNA+drug), which are cause hyperchromic.

Infrared radiation also used to study the interaction type between the drug and DNA, Figure (5), shows the IR results of the interaction between DNA and capecitabine

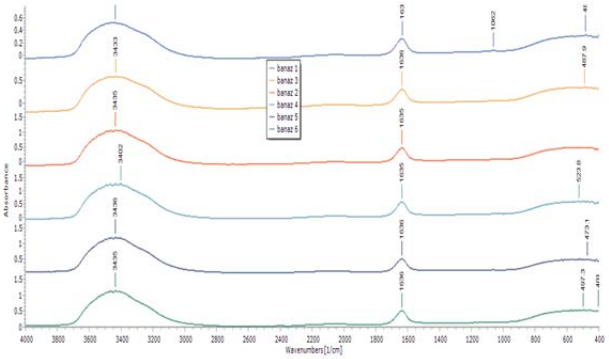


Figure (5): shows the IR results for five sample that contains drugs in the different concentration and DNA that extract from whole blood

Figure (6), shows the results of the IR for the DNA that are extracted from tissue. Which involve (free DNA and drug, in addition, include the IR results between DNA and drug" capecitabine" in the different concentrations.

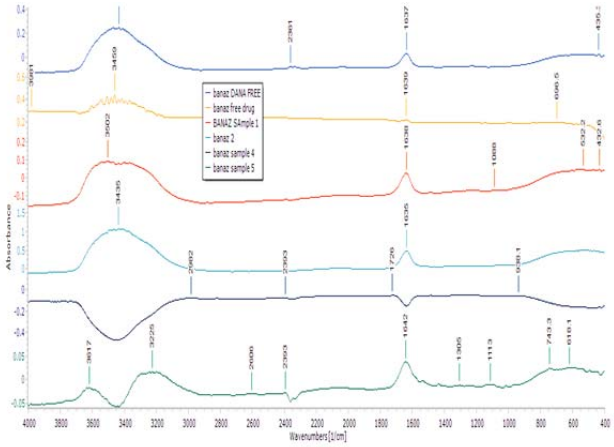


Figure (6): Shows the IR results for interaction between DNA that are extracted from tissue and five sample that are contains drug in the different concentration.

In both cases, we notice that a change in drug "capecitabine" leads to a change in the peck's location, shifts are noticed for many prominent IR bands of DNA when drugs are adding. For example, we notice that bands of Guanine exist in the 1713cm^{-1} that is a shift by (2-4 cm^{-1}) to 1711-1709 cm^{-1}. Bands of thymine are existed in 1669cm^{-1} which is shifts to 1662-1661 cm^{-1} as well as adenine bands at 1606 cm^{-1} in spectra of capecitabine-DNA complex.

4. Conclusions

This study represents a reliable approach for DNA extraction not only from fresh blood samples, also using frozen sample and applied the same Kit and method. When comparing the results of the DNA that are extraction by two methods, we find that for each method advantage and disadvantage, but regarding purification and time it is preferable to use the kit method and as for the quantity extracted DNA, it is preferable to use the manual method. As the present time, we find some companies are working to make some of the kits that the procedure of it are similar to what is in the manual method, in order to obtain the possible amount of DNA extracted. Results of the interaction shows that drugs have inter-mediate kind of intercalative properties and the binding is strong enough to interacted with vital DNA processes and could be helping in anticancer properties of the drug. In addition, this interaction effect on the conformation of the DNA especial B-DNA and cause perturbation.

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