The Effect of pretreatment and time of Storage on the determination of selenium by AAS in tissues of patients with breast tumors.

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Abstract

This study involves an investigation on the effect of pretreatment using many acids or their mixtures on the determination of selenium in a standard reference material of tissue.

The mixture ($HClO_4$: H_2O_2) (1:1) gave the best accuracy in the determination of selenium concentration in the standard sample, the optimum conditions for the other parameters (Temperature & time of storage of the tissue in solutions (formalin & normal saline) were also evaluated.

The standard calibration curve revealed a straight line and high sensitivity indicated by detection limit ($DL=0.3 \times 10^{-3}$ ug / ml.) and % recovery = 96.2 .

Selenium can be measured in tissue samples which kept in formalin up to 6 days for both Benign & malignant tissues . But, in normal saline up to 4 days in both benign & malignant tissues can be tolerated .

Higher values for selenium concentration in malignant tissue compared with benign tissue are found .

In the other hand lower values for selenium concentration in sera samples of patients with breast cancer are noticed

الخلاصة

تضمن هذه الدراسة التحرى عن تأثير المعالجات الأولية باستخدام عدد من الحوامض القوية أو مزيجاتها لقياس عنصر السلينيوم في المادة المرجعية الموثقة للنسيج.

أعطى المزيج ($HClO_4$:H_2O_2)(1:1) أفضل دقة عند قياس عنصر السلينيوم في المادة الموثقة المستخدمة في هذا البحث ,درست تأثير درجة الحرارة وفترة خزن النسيج في المحاليل الحافضة {الفورمالين والمحلول الملحي الطبي}كمحاليل حافضة للنسيج.

أعطى منحني المعايرة القياسي خـط مـستقيم ذو حـد كـشف مقـداره (DL:0.3*10^{-3}µg/ml) وبأسترجاعية مقدارها(Rec%:96.28),لوحظ أنه يمكن قياس عنصر السلينيوم في الأنسجة المحفوظـة فـي الفورمالين إلى حد ستة أيام فقط من فترة الحفظ في حالتي أنسجة الأورام الحميدة والسرطانية .أما في حالـة استخدام المحلول الملحي الطبي كمحلول حفظ ,فلوحظ أنه يمكن قيـاس عنـصر الـسلينيوم فـي الأنـسجة المحفوظة في المحلول الملحي إلى حد أربعة أيام فقط .

تم قياس عنصر السلينيوم في أنسجة وأمصال دم المرضى المصابين بأورام الثدي ولـوحظ زيـادة واضحة في أنسجة الاورام السرطانية عنه في الأنسجة الحميدة,بينما ينخفض تركيز السلينيوم فـي أمـصال المرضى المصابين بألاورام السرطانية مقارنة بالأورام الحميدة.

Introduction

Selenium is an essential trace elements its importance was only discovered in 1973.Morover the element is also known as a protective agent against heavy metal toxicity[1-3], cancer [4] and cardiovascular disease[5-8].

Its antioxidant role as selenoprotein in enzyme glutathion peroxidase is established [9].

Selenium glutathionine peroxidase acts as apart of antioxidant defense system of cells protecting lipids in cell membrane from the destructive effect of H_2O_2 and O_2^- generated by excess oxygen .

Most of the selenium in tissue is present in two forms selenocystien & selenomethionine [10-11] .

Selenium with too low concentration level causes deficiency disease ,the deficiency of selenium may increase cancer risk .

Because of the increasing interest of selenium as an essential trace element for human physiology ,classified methods for its determination stands as sensitive techniques were available , but the atomic absorption spectroscopy (graphite furnace) still remain attractive to analysis , because it is simplest and it provides experimentally most reliable test and more specific and sensitive indicator of selenium .

Material & methods

Instrumental apparatus

1- Atomic Absorption spectrometer type :"Electrothermal – AAS[Shimadzu AA 680G]" which consist of

a-Power supply (GFA-4B)

b-Graphite furnace (GFA-4B)

c- Auto sample changer(ASC-60G)

d- Graphite printer (PR-4)

2-Cell homogenizer MSK(W-Germeny),B.Braun cell homogenizer rotary –ball-mill type,Two-speed shaking action ,2000 and 4000rpm .

3- Lypholizer (GT_2):
Leybold-Heraeus (LH) (Germany).

4-Oven :
Heraeus type (MR-170E), temperature range(100-1000)^oC .

Chemicals :-

1-Selenium standard solution covering the range(0.05-0.3)µg/ml were prepared by the dilution of standard solution (1000 ug/ml)from selenium nitrate with deionized water and stored in tightly closed polyethylene bottels .

2- Many acids mixtures were prepared to digest the Certified Reference Material (C.R..M) these mixtures are : ($HClO_4$:HNO_3)(1:1),(1:2),(1:3),(1:4) and (1:5)
(HCl:HNO_3) as (1:4).

Many other acids & oxidative agents were used to digest the (C.R..M) in order to select the proper acid which gave the real

concentration of selenium in the certified reference material.

Sample Collection :-

In this study forty one (tissues & Sera) samples of patients with breast tumors were collected from Al-Kindy hospital, benign & malignant breast tumor tissues were obtained from the breast of individual at the time of mastectomy.

Methods :-

- **Preparation of samples :**

0.05- 0.2 gm of dry tissue was wieghed and transferred accurately to a round bottom flask and 1ml of concentrated acid or concentrated acid mixtures was added to the tissue sample and heated , filtered & diluted with deionized water to 25 ml .

Storing Effect :-

Tissue samples of six individuals were divided into three parts ,the first one stored in formalin (30%), but the second one stored in normal saline (0.9%) and the last one remain as it was .

Selenium concentration were measured in both tissue samples & storage solution after the storage time of 1,2,4,6,8,15, and 30 days using graphite furnace atomic absorption spectroscopy (GFAAS).

Results & discussion

1-Type of acid :

The acids HNO_3, (HNO_3:$HClO_4$)(1:1),(1:2),(1:3),(1:4) and (1:5),(HCl:HNO_3)(1:4) and the ashing methods at 450 ^oC and digestion by each individuals acid ratio (HCl:H_2O)(1:1),(HNO_3:$HClO_4$)(4:1) and (HNO_3:H_2O_2)(1:1) respectivily which were used for the digestion of (C.R.M)it was found these acids were not suitable for selenium determination , but the mixture ($HClO_4$:H_2O_2)(1:1) ensurs the complete conversion of selenium (+4) in to selenium (+6), our recommendation that this procedure for oxidation of selenium (+4)was much easier than the other previously mentioned methods .Table[1].

Temperature Effect :

The temperature (40,60,80,110,120,130, and 150) ^oC were applied on tissue samples of benign & malignant breast tumors in order to select the best temperature which leads to complete digestion of tissue samples & gives the best concentration value of selenium .The best concentration for selenium determination in tissue samples was obtained at 130-150 ^oC figure [1].

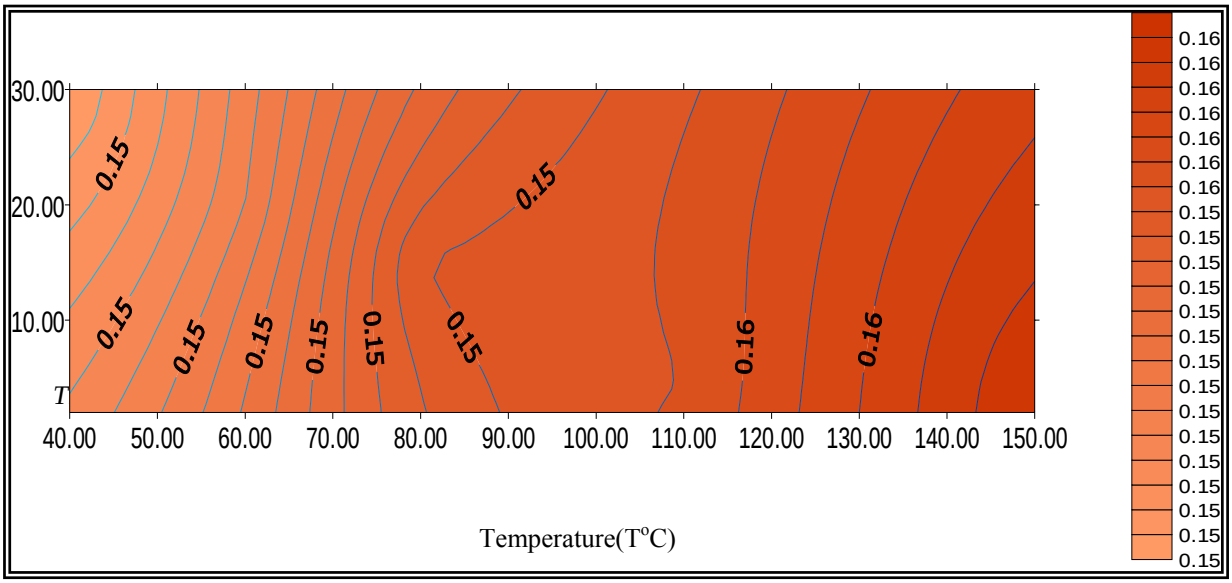
Storing Effect :

The tissue samples of (benign & malignant)tumors of six individuals which were stored in both storage solutions(formalin & normal saline) were used for the measurement of selenium under the calibration curve of selenium standard solution , using (GFAAS)after the storage times of (0,1,2,4,6,8,15,and 30 days).

Table[1]:Selenium concentration in certified reference material (MA-A1) by different digestion solution

Type of acid or its mixture	Se Conc. C.V=3.0 (ppm)			
	A.V (ppm) ± SD	E	E%	Rec% ± SD
Conc. HNO_3	2.70±0.21	-0.30	10.0	90.00±7.00
(5:1) (HNO_3:$HClO_4$)	2.47±0.06	-0.53	17.6	82.34±2.03
(4:1) (HNO_3:$HClO_4$)	2.63±0.24	-0.37	12.3	87.67±7.88
(3:1) (HNO_3:$HClO_4$)	2.30±0.14	-0.70	23.3	76.66±4.73
(2:1) (HNO_3:$HClO_4$)	2.21±0.06	-0.79	26.3	73.67±1.86
(1:1) (HNO_3:$HClO_4$)	2.01±0.03	-0.99	33.0	67.0±0.88
(1:1) (HNO_3:H_2O_2)	2.00±0.02	-1.00	33.3	66.66±0.57
(1:1) ($HClO_4$:H_2O_2)	2.93±0.05	-0.07	2.33	97.66±1.77
(4:1) (HNO_3:HCl)	2.06±0.06	-0.94	31.30	68.67±2.00
(1:1) (HCl:H_2O) after ashing at 450°C	1.96±0.03	-1.04	34.6	65.33±1.00
(4:1) (HNO_3:$HClO_4$) after ashing at 450°C	2.50±0.09	-0.5	16.6	83.33±3.06
(1:1) (HNO_3:H_2O_2) after ashing at 450°C	2.44±0.09	-0.56	18.7	81.33±2.91

C.V = certified value.
A.V = Analytical value
Rec% = Recovery %



Figure[1]:The effect of temperature on selenium concentrations in tissue samples.

In samples of benign tumors tissues (which were stored in formalin),the differences in selenium concentration were found as non significant ,except after 30 days, but the better result can be obtained after four days with (Rec%=94.45),but in malignant tumor tissue ,non significant decreasing in selenium concentration were noticed after all days under our study but, decreasing in recovery % of selenium concentration with increasing time of storage were noticed . Figure[2], our suggestion is that , the adsorption between selenium in tissue & formalin solution caused decreasing in selenium concentration in tissue samples .

So, from the result of recovery , good result for selenium concentration in tissue samples (which were stored in formalin) can be obtained up to six days only for both benign & malignant tissue , and the differences in selenium concentration in formalin solution were shown to be significantly increasing in selenium concentration values after six days ,Figure[3].

Using normal saline as a storage solution for benign & malignant tumor tissue , selenium concentration significantly decreased after 15 days with recovery % of 53.18 in benign and after six days in malignant tissue with recovery of 61.58, but good result can be obtained up to four day in both benign & malignant tumor tissue, this may caused by the effect of osmotic pressure ,the cell membrane was ruptured by the effect of this pressure which cause the emerging of all the cell contents to the storage solution (normal saline), in normal saline solution , there was non significant increasing in selenium concentration in normal saline of benign breast tissues , because of the decreasing in selenium concentration in their samples , but with important increasing in selenium concentration value in normal saline solution of malignant tissue after one day ,because of the increasing in its selenium content , Fig.[3].

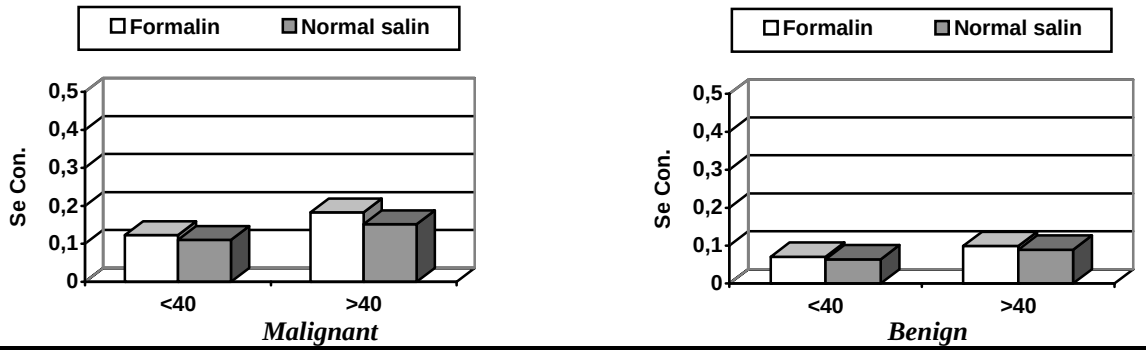
Forty one tissue samples of patients with breast tumors (benign & malignant)divided into two groups (<40)as a reproductive age and (>40) as a non-reproductive age were analyzed to determine selenium in it .High levels of selenium were obtained in non-reproductive age of patients with breast cancer compared with benign , these results were agreement with that reported by others[12-13] .Tab.[2].

Sera samples of the same patients with breast tumors were also collected to determine selenium concentration values in it using GFAAS.

It was found that selenium concentration decreased in sera of patients with breast cancer compared with benign with high significant differences , this result is in accordance with previous studies [14].Tab.[3]

Table [2]: Determination of selenium in tissue samples of patient with breast tumors.

Age group	Malignant			Benign			ANOVA	
	N	Mean ± SD		N	Mean ± SD		C.S	
		Formalin	Normal-salin		Formalin	Normal-salin	Fomalin	Normal-salin
<40	11	0.1234	0.1103	16	0.0706	0.0636	13.047 H.S	11.096 HS
> 40	9	0.1831	0.1521	5	0.0988	0.0890		

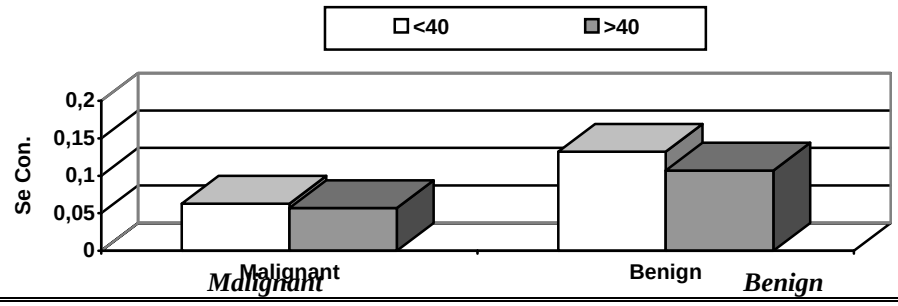


N=41, HS = highly significant

Table [3]: Selenium concentration in sera of patients with breast tumor.

Age group	Malignant		Benign		ANOVA
	N	Mean ± SD	N	Mean ± SD	C.S
<40	11	0.063±0.019	16	0.132±0.047	14.116 H.S
>40	9	0.057±0.016	5	0.107±0.020	

n = 20 **n=21**



HS: highly significant.

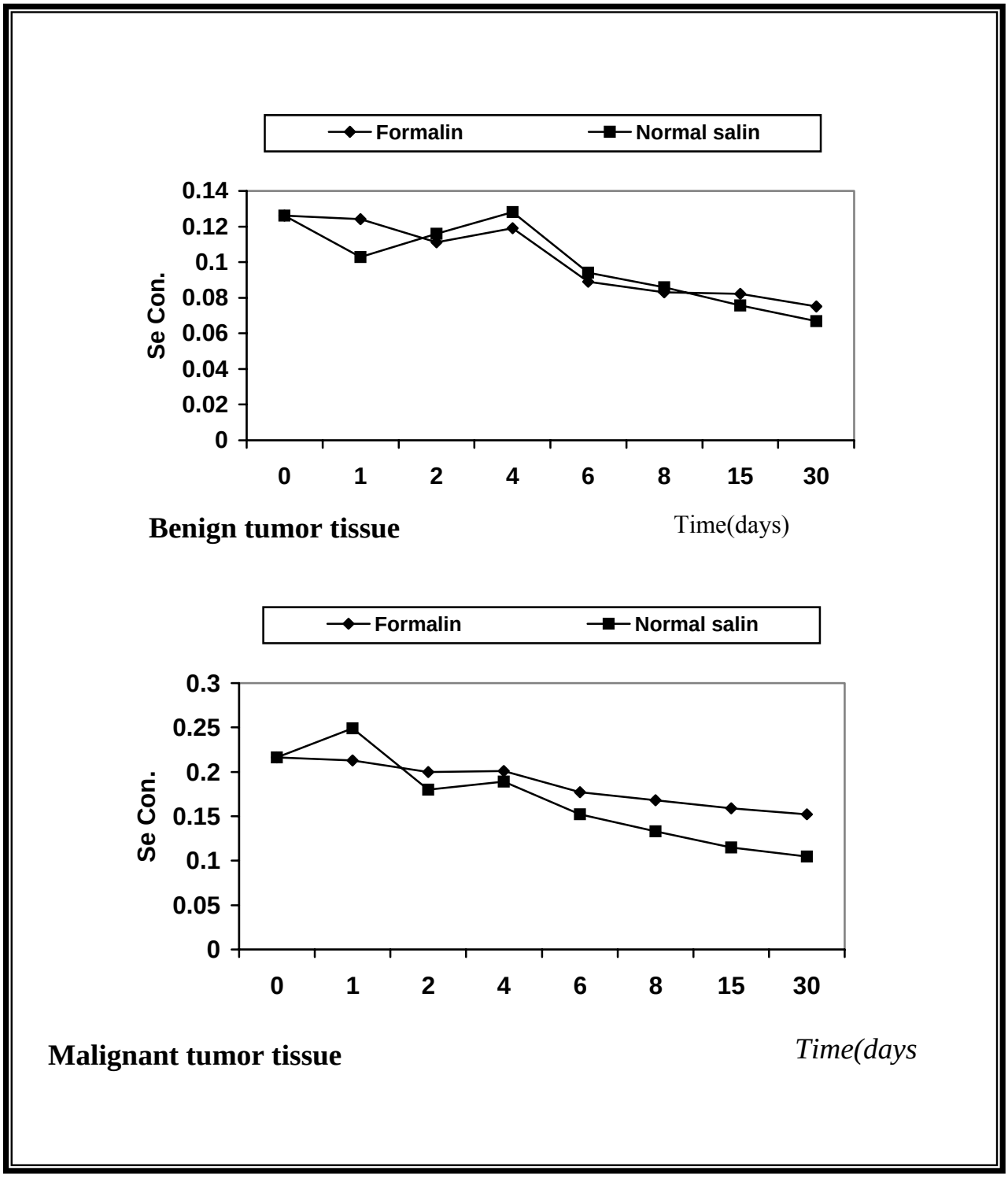
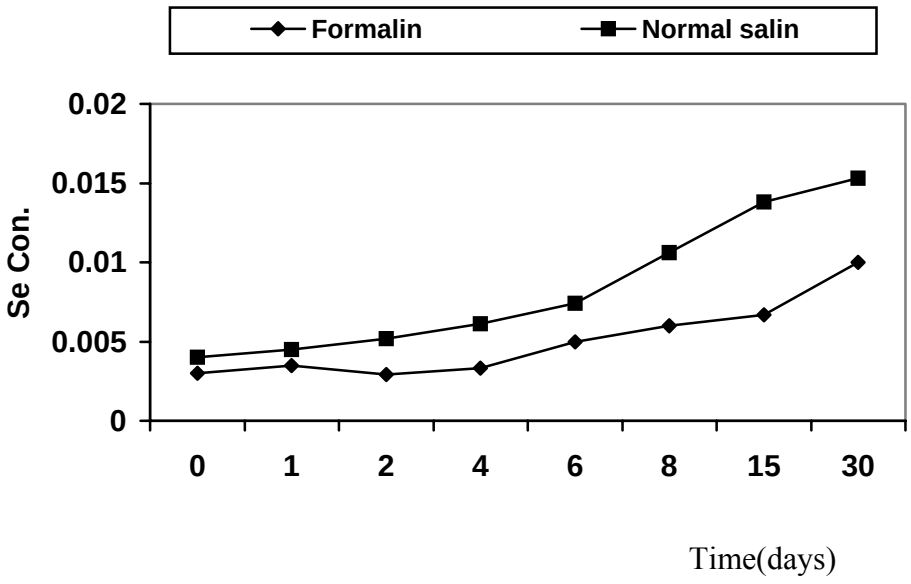
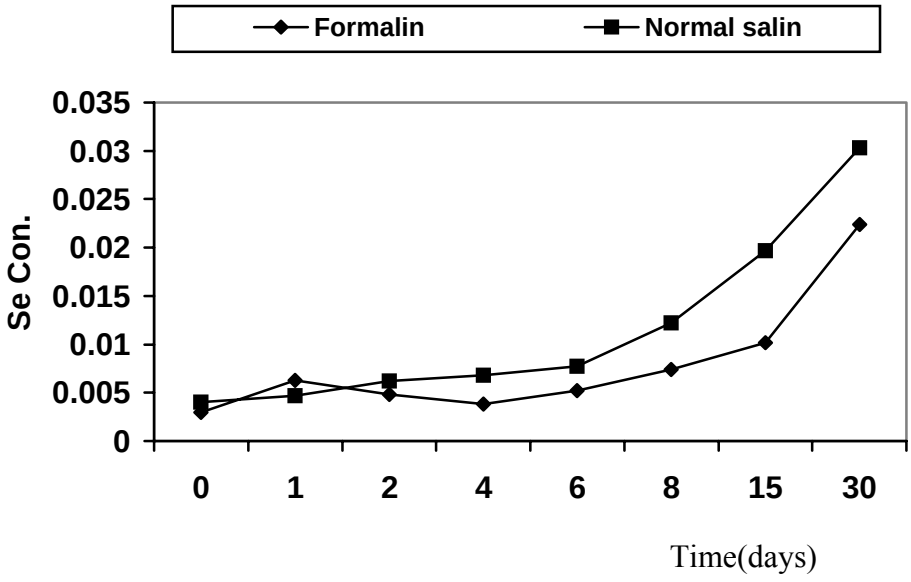


Figure [2]: The effect of time of storage in formalin and normal saline on selenium concentration values in tissue samples

Benign tumor





Malignant tumor

Figure[3]:The effect of storage time on selenium concentration in formalin & normal salin

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