Result &discusion

Biological Activity

Table (4a): Determination of the approximate acute median lethal dose (LD_{50}) of the tested compounds (37a and 37b).

Animals	The tested comp. (37a&37b) Doses (mg/kg)	Survival time
1	1.5	Non lethal effect
2	1.75	
3	2	
4	2.5	
5	3	
6	3.5	
7	4	
8	10	

When no deaths were recorded , the doses administered were increased as follows table(4b) :

Animals	The tested comp. (37a and 37a) Doses (mg/kg)	Survival time
1	30	Non lethal effect
2	50	
3	100	
4	160	

No deaths were recorded The tested compounds. (37a&37b)are safe up to 160mg/kg .

Table (5): Dose response of viable EAC cells (x 10^6 cell / ml) of the tested compound (37a)

groups / No. of animals	The tested compound (37a)					
	Tumor	**10mg/kg**	**30mg/kg**	**50mg/kg**	**100mg/kg**	**160mg/kg**
1	198	165	134	112	172	144
2	187	156	147	106	177	156
3	184	152	146.6	94	180	145.5
Range	(198 – 184)	(165 – 152)	(147-143)	(112 -94)	(180 – 168)	(156 – 144)
Mean ± SD	189.67 ± 7.37	157.67 ± 6.66	142.53 ± 7.39	104 ± 9.17	175± 6.24	148.5± 6.53
± SE	4.26	3.85	4.27	5.29	3.61	3.77
Significance P		<0.01	<0.01	<0.001	<0.01	<0.01

Table (6): Dose response of viable EAC cells (x 10^6 cell / ml) of the tested compound (37b)

Groups / No. of animals	The tested compound (37b)					
	Tumor	**10mg/kg**	**30mg/kg**	**50mg/kg**	**100mg/kg**	**160mg/kg**
1	198	144	128	94	168	137
2	187	137	125	87	159	133
3	184	149	128	84	150	134
Range	(198-184)	(149-137)	(128-125)	(94 -84)	(168-150)	(137-122)
Mean ± SD	189.67 ± 7.37	143 ± 6.66	127 ± 7.39	88 ± 9.17	159± 6.24	131± 6.53
± SE	4.26	3.48	1.00	2.96	5.19	4.58
Significance P		<0.01	<0.001	<0.001	<0.05	<0.05

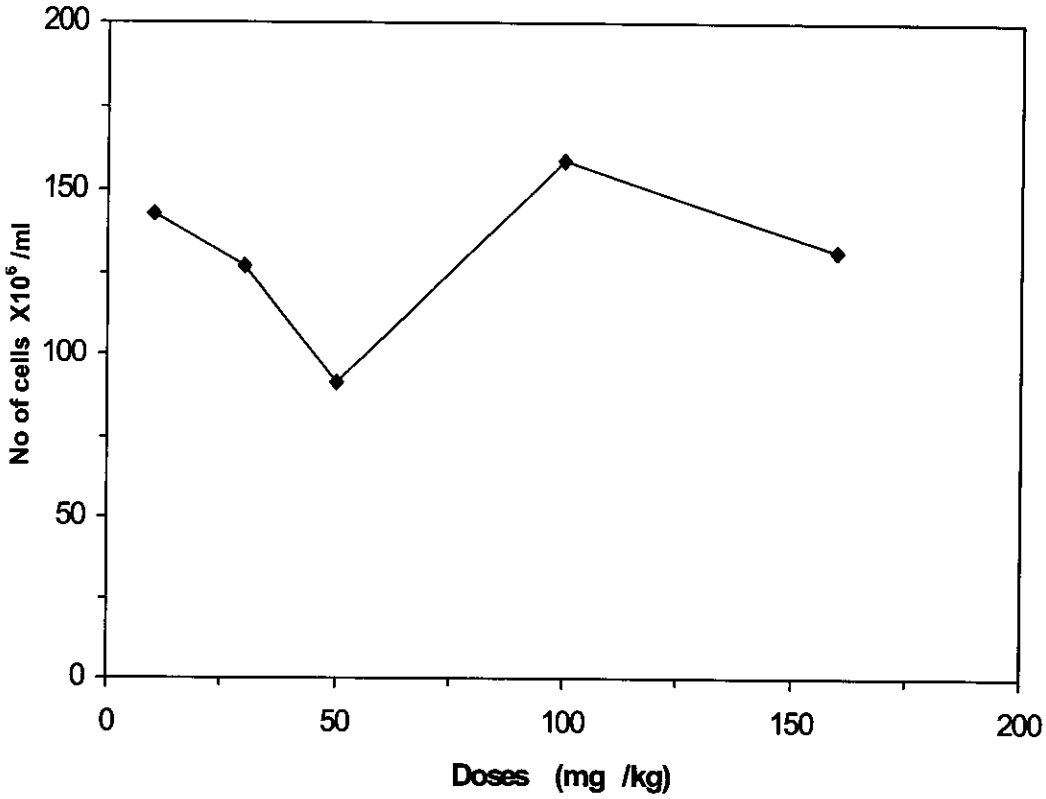


Fig. (10): Dose responses of viable EAC cells (10^6 cell/ ml) of tested compound (37a)

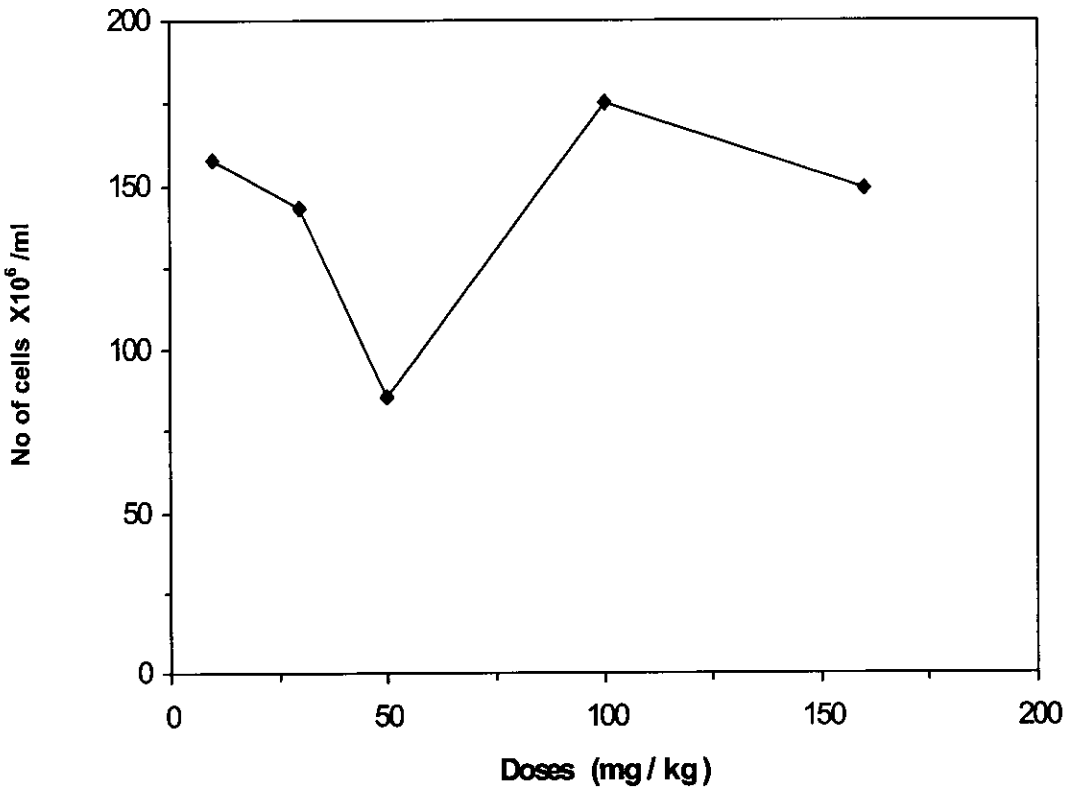


Fig. (11): Dose responses of viable EAC cells (10^6 cell/ ml) of tested compound (**37b**)

Table (7): Effect of Tested Compounds (37a and 37b) on the percent of change of body weight and life span prolongation of tumor bearing mice.

Groups / Survival period(days)	Tumor	Tested compound (37a)	Tested compound (37b)
2	0.8	0	0
4	3	0.3	0.6
6	12	1.5	5
8	25	6	8
10	31.9	8.5	10
12	36	11	15
14	33	15	22
16	48(1 dead)	23	26(1 dead)
17	52(2 dead)	25	30
18	55(3 dead)	27	33
19	56.5(1 dead)	31	34(1 dead)
20	52(1 dead)	30(1 dead)	38
21	50(2 dead)	33	40.5
22		38.5	41
23		41	43.5
24		42	41 (2 dead)
25		40.6(2 dead)	43
26		40.5	44
27		41.5	45.3
28		43(2dead)	46.5
29		45	48(2dead)
30		48(1dead)	50
31		42	45
32		39.5	45(1dead)
33		39(2dead)	42
34		38	41(2dead)
35		37(1dead)	40
36		34(1dead)	39.5(1dead)

Results are expressed as percentage mean body weight changes compared to the initial body weight at the beginning of the experiment.

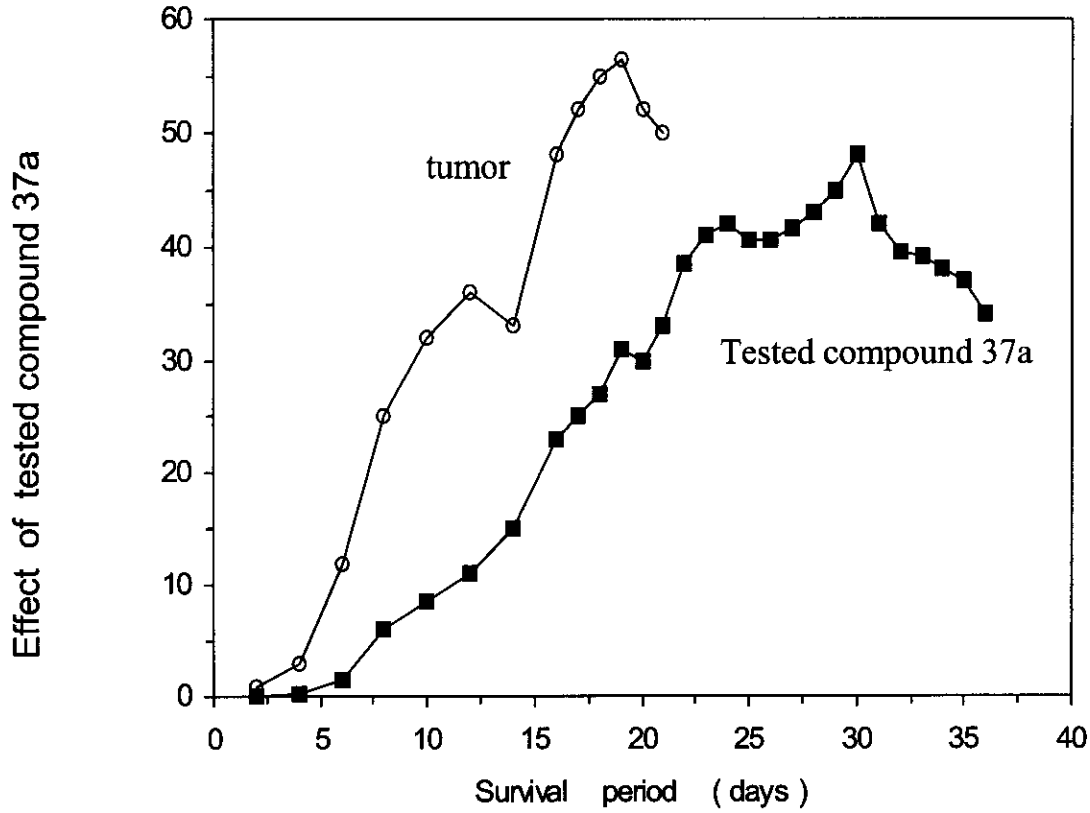


Fig. (12): Effect of Tested Compound (**37a**) on the percent of change of body weight and life span prolongation of tumor bearing mice.

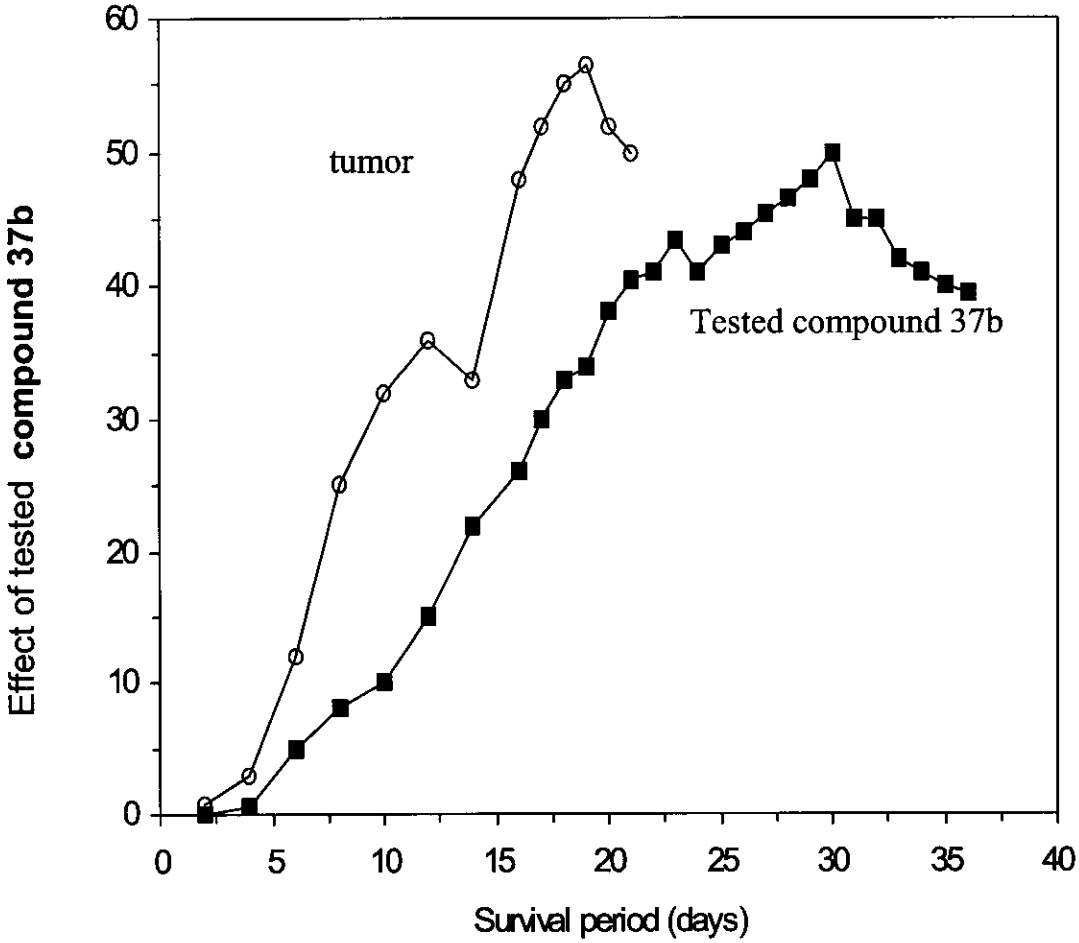
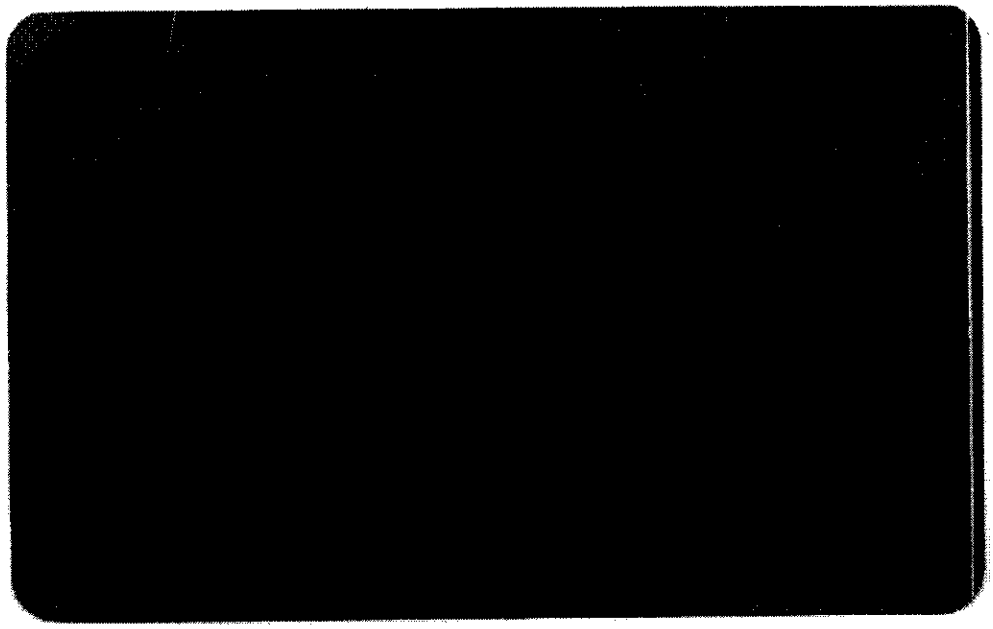


Fig. (13): Effect of Tested Compound (**37b**) on the percent of change of body weight and life span prolongation of tumor bearing mice.

Table (8): The antitumor activity of the tested comp. (37a and 37b) against Ehrlich ascities cells.

Groups		MST (days)	Survivors	T/C %
Tumor		18.5	-	100 %
Tested Comp. **37a**	Single dose	29.6	9/10	156.8 %
Tested Comp. **37b**	Single dose	27	8/10	145.9 %



Fig(14) Untreated (control) EAC Cells.

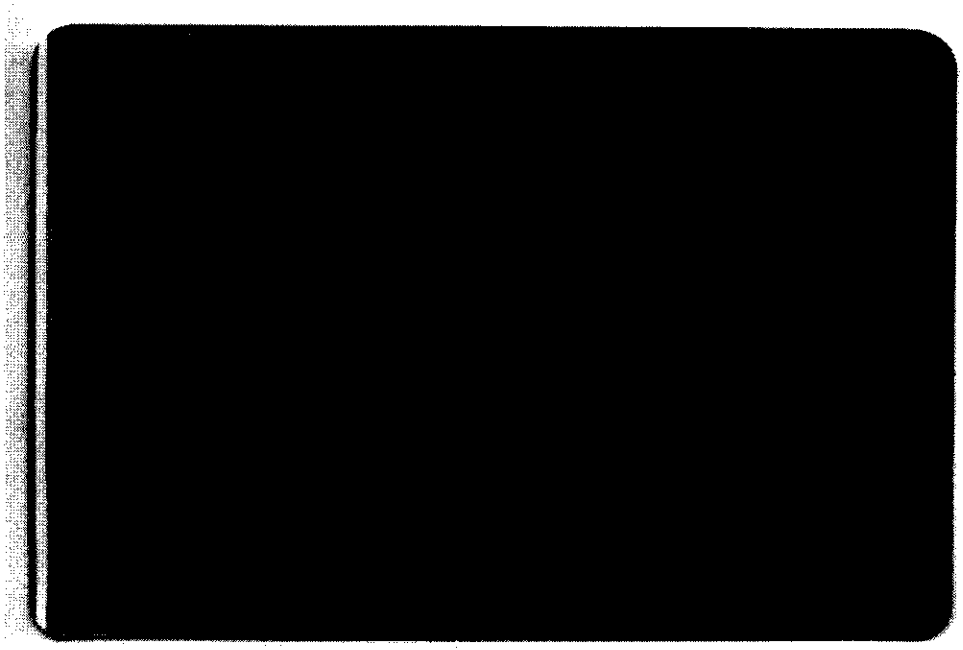


fig (15) photomicrograph of EAC cells treated with one injection of compound 37a

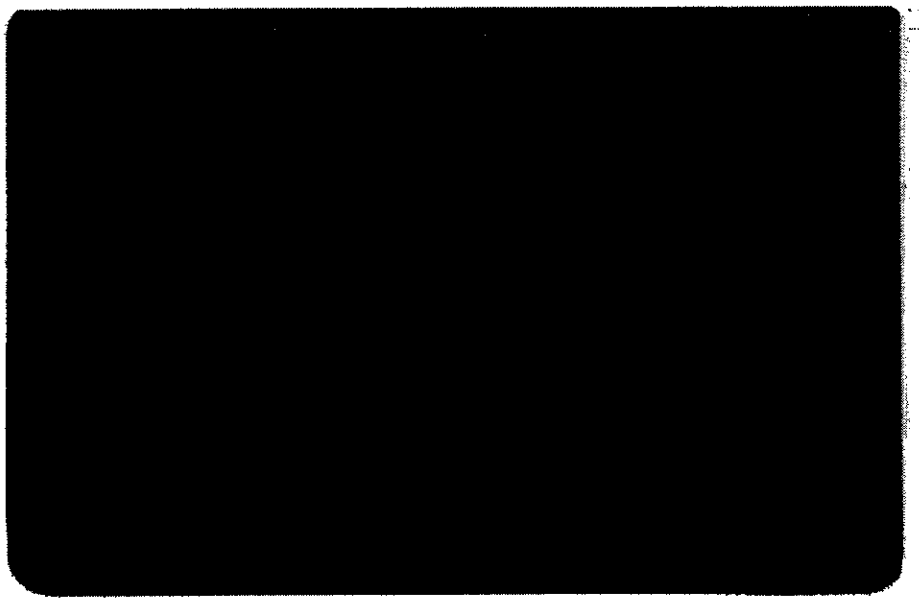


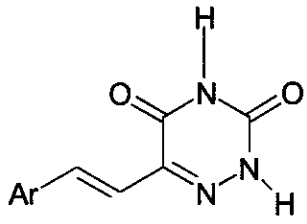
fig (16) photomicrograph of EAC cells treated with one injection of compound 37b

Biological activity:

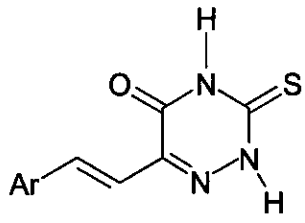
Search for new anticancer drugs is still feverwish. In all forms of drug therapy, clinicians must ensure that the maximum therapeutic benefit is achieved without non acceptable toxicity therefore, it is completing to search for new antitumor agents with different modes of action.

Numerous publications describe the synthesis of triazines possessing a variety of pharmacological activities such as antitumor **(Ivin et al., 1978),** antiviral **(Sanders, 1982),** fungicidal, **(Misra et al., 1979)** and herbicidal **(Jochem and Bayer, 1985).**

The tested compounds **37a** and **37b** were synthesized as mentioned before. The tested compounds **37a** and **37b** were selected due to the biological activities of triazines.



37a



37b

The tested compounds were dissolved in dimethyl sulfoxide (DMSO) concentration lower than 0.5%. DMSO improve the solubility of tested compounds and can influence better diffusion through membranes .Also DMSO does not affect tumor growth (**Andreani et al. 1983).**

Toxicity studies were carried out according to the method of **Meier and Theakston(1986).** The approximate acute lethal dose LD50 of the tested compounds **37a** and **37b** were tested in female Swiss albino mice administered i.p., from **table 4**a. It is clear that the tested compounds **37a** and **37b** were found to be non lethal at doses 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 10 mg/Kg and no deaths were recorded by increasing the doses administered to 30, 50, 100, 160 mg/Kg. The tested compounds **37a** and **37b** were considered to be safe up to the dose 160 mg/kg **(Table 4 a, b).**

Our results are in accordance with **(Murink and Nash, 1977; Pinter et al., 1990; Goldman, 1994)** who reported that triazine herbicides are generally characterized by low acute toxicity, these herbicides have been known to have limited mutagenic or carcinogenic activity.

Yaguchi et al., **(2006)** found that a new triazine ZSTK474 had strong antitumor activity against human cancer xenografts without toxic effects in critical organs at a concentration 1 micromole (1mM).

The dose response curves as shown in **Figs. (10&11)** were performed for the tested compounds **37a** and **37b** to determine the most effective dose for the inhibition of Ehrlich ascities carcinoma (EAC) cell growth. This experiment was carried out by administration of the tested compounds **37a** and **37b** at doses 10, 30 , 50, 100, 160 mg/Kg, after **24** hours from implantation of 2.5 $\times 10^6$ Ehrlich ascities tumor cells to female Swiss mice (average weight 22 ±2 g). Ten days after tumor inoculation, the animals were sacrificed, the ascities fluid was collected and the viable tumor cells were counted.

Results present for the tested compounds **37a** and **37b** in tables **5** and **6** and **Figs. 10,11** showed that the tested compounds **37a** and **37b** at dose 50 mg/Kg were exhibited the most profound effect on the inhibition of tumor cell growth. It caused a significant reduction of about **53.6%** ($P < 0.001$) for the tested compound **37b** and a significant reduction of about **49.16%** ($P < 0.001$) for the tested compound **37a** of the viable tumor cell counted, compared to the negative control (untreated group).

Variously substituted 2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine derivatives caused considerable growth inhibition on distinct tumor cell lines, 2-Amino-6-bromomethyl-4-(3,5,5-trimethyl-2-pyrazoline)-1,3,5-triazine showed the most potent antitumor activity **(Brzozowski and Sa˛czewski, 2000)**.

Also 2-[2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(5-nitro-2-thienyl) acrylonitrile show highly potent activity against some cell lines of Leukemia (CCRF-CEM, K-562, RPMI-8226, SR), CNS Cancer (SF-539) and Breast Cancer (T-47D) **(Brzozowski and Saczewski 2002).**

Maeda et al., (2000) reported that in the cytotoxicity test *in vitro*, 4PyDAT(2,4-diamino-6-(pyridine-4-yl)-1,3,5-triazine) showed very weak direct cytotoxicity against the Co26Lu cell line, Co26Lu(F55). Less microcapiral formation on tumors were observed for the treated group with a hemorrhage than the control group under microscopy. 4PyDAT significantly inhibited the production of urokinase-type plasminogen activator (u-PA) in Co26Lu(F55) cells. These results suggest that the antimetastatic and antitumor activities of 4PyDAT are due in part to inhibition of angiogenesis, rather than direct antiproliferative action on the tumor cells. 4PyDAT may become a lead

compound to develop antitumor triazine derivatives based on antiangiogenic action .

As to life span prolongation test from the results presented in **table 7** it is evident that the single dose for the tested compounds **37a** and **37b (figs. 12& 13)** exhibited the largest inhibitory effect on tumor growth as presented by the increased survival time of tumor bearing mice. This survival time reached 36 days. compared to 21days for untreated tumored group(negative control). Moreover, the same doses showed the lowest percent of change increased of body weight of tumored mice which indirectly represents tumor growth. These increases were recorded **49.16% & 53.6** for single dose of compounds **37a** and **37b** respectively. Compared to untreated group (tumored) .

T/C ratio was defined as the ratio of the mean survival time in days of the tested group divided by that of the control group and expressed as percent. It was found to be **149.9 %** and **156.8%** for the tested compounds **37a** and **37b** respectively **(table 8)** . Significant antitumor activity is achieved with the increase in life span by **25% (Andreani et al., 1983).**

Sakuria, (1964); reported that the criteria used for evaluating the tumor - inhibitory effect of a compound include ; inhibition of tumor growth , histological change (survival time of the host).

Lawrence et al., (1977) ; reported that the philosophy underlying all cancer treatment is to improve the quality and /or length of life of patient.

Also, **Livingston and carter,(1982) ;** reported that the object of clinical research in oncology is to improve both survival and quality of life of patient with cancer.

Due to foregoing studies, life span prolongation of female swiss albino mice and their quality were taken as preliminary parameters to elucidate the validity of the chosen biological compounds in treatment trials of EAC.

Cytological and Histological Effects.

The cytological and histological changes caused by cytostatic drugs are important factors of antitumor action . These can only be investigated if the drug under investigation causes histological changes.

Cytomorphological changes in the EAC withdrawn (in the 10th day after EAC inoculation) from mice treated with one injection of both compounds **37a,b** show significant celluler damage **(figs 14 - 16)** .

The major effect is demonstrated as disruption of the cytoplasm accompanied by marked rupture in the cytoplasmic membrane . Also, unequal nuclear division , formation of polynucleated and giant cells, and scattered chromosomes are shown in treated EAC **(figs 15& 16)** And confirming our foregoing results concerning life span prolongation and cell viability .These studies show clearly and confirm that 6 and 2-chlorophenyl vinyl triazines **37a,b** have inhibitory effect on tumor in EAC – bearing mice and arrest cell division.

A considerable interest in the mechanism of action of cytotoxic agents at the cellular level has been generated .

Our results are parallel to that of many authors .

Bruce et al., (1966) ; reported that some lethal agents may be regarded as cell cycle specific , or even phase specific within the cycle , while others act on resting non – cycling cells to the same extent as on cycling cells.

The grouping of agents according to their biochemical actions is in no way meant to imply that a given drug has only one biochemical mode of action or that the biochemical mode of action under which the drug is listed is necessarily related to its lathal or blocking action on cells . A single agent may have various biochemical , chromosomal , or mitotic effects which may not be related to their effect on cell viability **(Jones and Mauro, 1976).**

Clark (1975) ; reported that cytotoxic agents attack proliferating cancer cells at different stages of the cell cycle . They inhibit the synthesis of DNA, RNA and / or protien .

The ribo nucleoside of 4-amino-7H-pyrazolo[3,4-*d*]-*v*- triazine is a good substrate for adenosine kinase . Also have cytotoxic effect on growth-inhibition and potential antitumor agent. **(Bennett et al., 1976)**

A compound which inhibts DNA synthesis will either prevent cell from initiating DNA replication so that they remains in the late "G" ,

phase , or will reduce the rate of replication , so that they remain in the "S" phase .

DNA damaging agents have historically a central role in cancer therapy (**Foye, 1995**).

The large number of known DNA damaging agents can be divided into a relatively small number of categories if they are classified on the basis of functional groups and chemical reactions involved in their reactions with DNA damaging agents include enediynes, epoxides, imines, activated cyclopropanes, heterocyclic N-oxide and quinines (**Gates, 1999**) or the activity of the hydroxyl radical (**Ganely et al., 2001**).

Lu et al., (1980); reported that , the high potential activity of the 5-azacytidine(1-β-D-ribofuranosly-4-amino- -triazine -2(1)-one) against tumor cells were suggested to be related to treatment of animals with triazines lead to a specific reduction of the 5-methylcytidine (m5C) content of tRNA in tissues, e.g. liver, hepatoma, mammary tumor, and spleen; however, the effect of the drug on post-transcriptional tRNA modification was more general in lymph nodes and in leukemic cells. Many base modifications of L1210 cell tRNA were inhibited, with m5C and dihydrouridine (hU) being the most affected. A positive correlation

existed between the extent of inhibition of the formation of m5C and other modified nucleosides and the antitumor activity of the drug.

The activity of the tested compound **37b** was higher than tested compound **37a** which may be related to the presence of the sulpher in tested compound **37b.** The activity of the tested compound **37b** may be related to the presence of the sulpher. In general , sulpher containing compounds are known to antagonize the toxic effects of the drugs .**Weisberger and Storaasli, (1954);** reported that the active principle of garlic (allin) can inactivates many sulfhdrayl (- SH) enzymes and can react rapidly with cysteine . This compound may be of importance in relation to malignancies because of the availabity of the reduce – SH compounds which often been implicated in the processes of the cell growth and division . The importance of -SH compounds in these processes is supported by the demonstration of a high –SH content in proliferating tissues and the inhibition of cell division by thiol poisons such as alkylating agents and heavy metals **(Baron, 1949 ; Brachet,1950 ; Contoplus and Anderson, 1950).**

The National Cancer Institute has spent in excess of $20 million over the past several years researching and identifying plant substances in foods and herbs that provide protection against cancer and other

diseases. These substances are called phytonutrients and phytochemicals. allyl sulfides in garlic and onions; Found to posses Antimutagenic, anticarcinogenic, immune and cardiovascular protection. anti-growth activity for tumors, fungi, parasites, cholesterol and platelet/leukocyte adhesion factors. They boost the immune system, assist the liver in rendering carcinogens harmless, and may reduce production of cholesterol in the liver.Specific allylic sulfides block the activity of toxins produced by bacteria and viruses.

Also the presence of the chloro group in compounds **37a** and **37b** improve biological effects and binding to DNA this is in parallel with **(Roloff et al., 1992; Meisner et al.,1993)** reported that Chlorotriazine herbicides (atrazine) [2-chloro-4-ethylamino-6-isopropylamino-s-triazine] is concerned with DNA and chromosome damage.

We suggested that the results of the present study indicates that the 6 and 2-chlorophenyl vinyl triazines 37a,b have significant cytotoxic activity. It is believed that our results may lead to the development of antitumor agent.