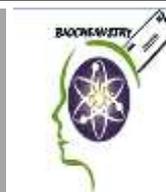




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Anti Cancer effect of two new synthesized hetero cyclic compounds.

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ABSTRACT

Background: Thiazole derivatives are a category of hetero cyclic compounds that large spectrum of biological activities.**Aim:** This study aim to study the anti cancer effect of thiazole derivatives invitro and invivo.**Methods:** Synthesis of thiazole derivatives were examined *invitro* and *invivo* .**Results:** *Invitro* , IC50 of Compound1 on different human lines colon cancer, breast cancer and liver cancer were found 4.7 ug/ml, 4.8 ug/ml and 11 ug /ml respectively. While IC50 value for Compound 2 were found 9.5 ug/ml , 9.6 ug/ml and 18 ug /ml respectively. *Invivo* , these compounds were safe up to 500 mg/Kg.Although the most effective doses was 15 mg/kg for Compound1 and Compound2. Thiazole derivatives showed a significant reduction in the volume and count of Ehrlich ascites carcinoma and improve hematological parameters ,liver and kidney functions.**Conclusion:** Thiazole derivatives have apotent antitumor activity .

I

I. Introduction:

Cancer still represents one of the most serious human health related problem; despite the great progress in understanding its biology and pharmacology. The usual therapeutic methods for cancer treatment are individually useful in particular situations and when combined with other remedies, they offer a more efficient treatment for tumors (1).Thiazole ring is a structural fragment of natural compounds such as thiamine (vitamin B1), thiamine pyrophosphate (TPP, a coenzyme important in respiration in the Krebs cycle), epothilones, carboxylase, and the large family of macrocyclic

thiopeptide antibiotics, thiostrepton and micrococcin P1 (2). Thiazole derivatives are associated with a broad spectrum of biological properties, including anti-convulsant. Antagonists with anti-thrombotic activity, as new inhibitors of bacterial DNAgyraseB (3). This work study the antitumor effects of two new compounds of thiazole derivatives The **first. compound:** 5- (*p*- chloro phenyl) - 2- [(ethoxy- carbonyl- methylidene) - hydrazino]- 1, 3- thiazole.

Second Compound: 2- [5- (*p*- chloro phenyl)- 1, 3- thiazole- 2- yl]- 1- [(ethoxy carbonyl), (5- *p*- chloro phenyl

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- 1, 3- thiazole- 2- yl- amino) methylidene] - hydrazone.

II. Material and Methods:

Chemicals:

The chemicals and solvents were obtained from Al drish chemicals Company, Kits were from Biodiagnostic Company.

Synthesis of Thiazole derivatives.

The synthesis of the thiazole derivatives (CP1&CP2) Preparation of ester glyoxal thiosemicarbazone was obtained via the condensation of ester glyoxal with thio semicarbazide, as a key starting material, according to the method described by **Minh**(4). Cyclization of thio semicarbazone derivatives with *p*- chloro phenyl bromide in ethanol in the presence of fused sodium acetate under reflux to yield the corresponding 5- (*p*- chloro phenyl)-2-[(ethoxy carbonyl methylidene)- hydrazino]-1,3-thiazole by **Elsayed and Zaki** (5),(6). Condensation of 2-substituted hydrazino - 1, 3 - thiazole with 5 -(*p* - chloro phenyl)- 2- amino- 1, 3- thiazole in di methyl formamide under reflux, was expected to give 2- [5- (*p*- chloro phenyl)- 1, 3- thiazole - 2- yl]- 1- [(5 - *p* - chlorophenyl)- 1, 3- thiazole- 2- yl amino carbonyl) methylidene]-hydrazone via nitrogen nucleophilic attack at carbonyl function of ester group of these compound, but give 2- [5-(*p*-chloro-phenyl)-1, 3- thiazole- 2- yl]- 1- [(ethoxy carbonyl), (5-*p*- chloro phenyl-1,3 -thiazole-2-yl-amino)methylidene]-hydrazone was yield, which may be formed via nitrogen nucleophilic addition at azomethene (CH=N) of CP1, followed by dehydrogenation **Mostafa** (7). Melting points were determined in open capillaries using electrothermal digital melting point apparatus and were uncorrected HNMR spectra were run with a Bruker 400MHz DRX-Avance NMR spectrometer. Compound were dissolved in DMSO as solvent. The IR data were obtained with Shimadzu 470 spectrometer. Micro analysis were

conducted using an elemental analyzer 1106.

Invitro study: The cytotoxic effects of CP1 and CP2 were carried out on different human cell lines as, HCT116 (colon cancer), MCF-7 (breast cancer) and HEPG2 (liver cancer) were evaluated by determining of IC50 values at The National Cancer Institute, Cairo, Egypt according to **Ferrari** (8).

In vivo study:

Animals:

Adult female Swiss albino mice weighed 22-25 g were purchased The National Cancer Institute, Cairo, Egypt used throughout this study. The animals were housed in steel mesh cages (animal house, faculty of Science, Zagazig University) and maintained on a commercial pellet diet for one week before starting the experiment as an adaption period.

Determination of median lethal dose LD50 of thiazole derivatives.

Approximate LD50 of two synthetic compounds were determined according to method of **Meier** (9).

Dose response curve. Determination of the most effective dose according to method of **Crump** (10).

Experimental design:

Total number of 120 female Swiss albino mice weighed 22-25g was divided into the following groups (20 mice for each group) as follows:

Group (I): Negative Control: mice injected I.P. with Olive Oil three times for 10 days

Group (II): Positive Control (EAC bearing group): mice were injected with Ehrlich ascites carcinoma (EAC), (2.5×10⁶ cells) by I.P. injection once.

Group (III) compound 1 group: mice were injected I.P. with compound 1 (15 mg/Kg three times for 10 days).

Group (IV) compound 2 group: mice were injected I.P. with compound 2 (15 mg/Kg). Three times for 10 days.

Group (V) compound 1 treated group: mice were injected I.P. with compound 1

(15 mg/Kg) after EAC injection (2.5×10⁶ cells/mouse), followed by I.P. injection of compound 1 of EAC injection three times in 10 days.

Group (VI) compound 2 treated group: mice were injected I.P. with compound 2 (15 mg/Kg) After EAC injection (2.5×10⁶ cells/mouse), followed by I.P. injection of compound 2 of EAC injection three times for 10 days.

Samples collection:

At the end of the experiment, the blood samples were collected from the retro-orbital venous plexus under light ether anesthesia(11). Serum was prepared by centrifuging blood at 4000 r.p.m. for 10 minutes. Serum was aliquot and stored at -20 °C until biochemical analysis .EDTA vacuum tube which run in a hematology analyser for estimating hematological parameters in experimental mice groups by using Automatic CBC analyzer. Ehrlich ascites carcinoma (EAC) cells were harvest from each mouse in centrifuge tube containing heparinized saline. Note the volume of ascetic fluid in each mouse in each group. Each sample of cells were undergoes counting and viability of EAC cells and apoptotic parameters in each mouse in each group. Liver and kidney tissues from each group were collected and preserved in Formalin solution until histological examination and part of the liver and Kidney was also collected and preserved in phosphates buffer saline (pbs) (PH 7.4) for tissue homogenate preparation (12).

Methods: Viability and Counting of EAC cells: determined by the Trypan Blue Exclusion Method (13),

Life span prolongation.Life span calculated according to the method described by Mazumdar (14).

Determination of hematological parameters.Hemoglobin(HB),red blood cell (RBCs), white blood cell (WBCs), platelets counts and blood indices were determined using blood hematology

analyzer using Automatic CBC analyzer (Sesmex Kx-21).

Determination effect on liver and kidney. Alanine amino transferase (ALT) (15), aspartate aminotransferase (AST) (16), albumin (Alb.)(17), Bilirubin (bili) (18)creatinine (Cr)(19), urea (Ur)(20), were determined in serum.

Statistical analysis:

All statistical analyses were done using Statistical Package for the Social Sciences (SPSS) software version 22.The obtained data were expressed as M±SE. Independent student-t test was performed to detect the significance of the treated groups compared to control. In addition, one-way ANOVA test was performed to compare means of all groups (21).

Results

Solubility: These compounds were dissolved in dimethyl sulfoxide (DMSO) used as organic solvent. And dissolved in Olive oil.

Results of chemical analysis:

The first compound (CP1): **5 - (p-chlorophenyl) -2 -[(ethoxy carbonyl methylidene) hydrazine]-1,3 - thiazole.** Pale yellow crystals; yield 72%, m.p.147 OC. IR (KBr): v_{max}= 3201 (NH), 1697 (C= O), 1635 (C= N), 1605, 1550 (C= C), 1053, 1010 (C- O) cm⁻¹.

¹H- MNR (DMSO - d₆) δ: 1.28 (t, 3H.CH₃), 4.24 (q, 2H, OCH₂), 7.38-7.91 (m, 6H ,Ar- H, H-thiazole and CH=N), 12.88 (br.S. 1H, NH) ppm.

Anal.Calcd for C₁₃H₁₂N₃ClO₂S (309): C, 50.48; H, 3.88; N, 13- 59 Found: C, .27 ; H , 13.3, as shownin Fig. (1,2).

The second compound (CP2): **2-[5-(p-chloro phenyl)- 1, 3- thiazole- 2- yl]- 1-[(ethoxy carbonyl), (5-p-chloro phenyl) 1,3- thiazole- 2- yl-amino) methylidene]- hydrazine.** Pale Yellow crystals, yield (67%).M.p. 125 OC. IR(KBr): v_{max}= 3201, 3105 (NH), 1697 (br.C= O), 1627 (C= N), 1605, 1543 (C= C), 1091, 1053 and 1010 (C-O)cm⁻¹.HMNR (DMSO- D₆) δ: 1.27 (t, 3H, CH₃), 4.22 (q, 2H, OCH₂), 7.28- 7.92

(m, 10H, Ar- H, and H-thiazole, 12.43 (S, 1H, NH), 12.91 (br.S, 1H, NH) ppm. AnalCalcd for $C_{22}H_{17}N_5Cl_2O_2S_2$ (518): C, 50.96; H, 3.28; N, 13.51. Found: C, 50.78; H, 3.03; N, 13.33, as shown in Fig.(3, 4).

Docking score:

An trial to study the structure–activity relationship using the molecular docking software for determination the binding affinity of the synthesized compounds for the p53 protein (PDB: 1TUP). the synthesized compounds showed high binding affinity toward the target p53 protein with docking score (-5.67 kcal/mol) and making two hydrogen bonds, one hydrogen bond with Glu 285 and another one with LYS 132 (as shown in fig. 5 , fig. 6).

In vitro study: The cytotoxic effect of CP1 and CP2 on human cancer cell lines was shown in figure (7-12) were evaluated by determining of IC50 values. The IC50 values of CP1 on HCT116 (colon cancer), MCF-7 (breast cancer) and HEPG2 (liver cancer) found 4.7 ug/ml, 4.8 ug/ml and 11 ug /ml respectively. While IC50 value for CP2 found 9.5 ug/ml, 9.6 ug/ml and 18 ug /ml respectively. ,as shown in Fig.(7-12).

The in vivo results:

The toxicity: Determine the median lethal dose (LD50) of thiazole derivatives our results revealed that, doses up to 500 mg /kg were considered safe for thiazole derivatives, where no mortality was observed.

Dose response curve: The most effective dose of thiazole derivatives reduced the number of EAC cells was found to be 15 mg /kg,as shown in Fig.(13,14).

Viability: Thiazole derivatives with the positive control group, where volume and count of tumor cells, we find that ascites inhibition rate increased and Volume decreased to reached 50.7% in CP1 Therapeutic and 53.4% in Cp2 Therapeutic. The count of tumor cells in ascites tumor cell in the Cp1

Therapeutic & Cp2 Therapeutic groups were lower than of the control group by 77.5% and 74.5% respectively compared to positive control group. ,as shown in Fig.(16,17).

life span prolongation: The mean life span prolongation in positive control group was found to be 16 days,while treated groups of thiazole derivatives showed a significant increase in the life span prolongation to 20 days by 25% (T/ C ratio = 125 %), in CP1 and 22 days by 37.5% (T/ C ratio = 137.5%), with CP2, compared to positive control group. ,as shown in Fig.(15).

The effect of CP1 and CP2 on hematological parameters:

RBCs count increased in CP1 and CP2 groups by 18.3% and 15.2% respectively compared to positive control group, also hemoglobin concentration increased by 35.5% ,19.2% respectively compared to positive control group. In addition, administration of CP1 and CP2 raised WBCs count by 125.5% and 37.2% respectively compared to positive control group. Platelet count increased by 19.4%, 23.4 %; respectively compared to positive control group. ,as shown in Table.(3).

Effect of CP1 & CP2 on Liver functions in all studied groups:

The mean value of AST activity was found to be (139±2.88 U/L) in negative control group, positive group showed a significant increase in AST activity to be (237 ±1.15 U/L) by 70.5%,(p<0.001), compared to negative control group. While CP1Th&CP2Th showed a significant increase to (259±0.57 U/L), (272±1.73 U/L) by 9.2%, 14.7%; respectively (p<0.01), compared to positive group. Also, CP1&CP2 groups showed a significant increased to (205±1.15 U/L) (217±1.73 U/L), by 47.4% 56.1%; respectively (p<0.001), compared to negative control group. While, mean value of ALT level was found to be (45±1.15U/L) in negative control group, positive group

showed a significant increase in ALT activity to be (62 ± 1.15 U/L) by 37.7 %, ($p < 0.001$), compared to negative control group. while Cpd1 therapeutic & Cpd2 therapeutic showed a significant decreased to (43 ± 1.15 U/L), (37 ± 1.73 U/L) by -30.6%, -40.3 %; respectively ($p < 0.001$), compared to positive group, also, Cpd1 & Cpd2 groups showed a significant increase to (70 ± 2.88 U/L), (57 ± 1.73 U/L), by 55.5 %, 26.6%; respectively ($p < 0.001$), compared to negative group. Also, mean value of ALB level were found to be (3.4 ± 0.05 g/dl) in negative control group, positive group showed a significant decrease in ALB level to be (2.3 ± 0.05) by -32.%, ($p < 0.001$), compared to negative control group. while Cpd1 therapeutic & Cpd2 therapeutic showed a significant increased to (3.6 ± 0.05 g/dl), (3.5 ± 0.05 g/dl) by 56.5%, 52.2%; respectively ($p < 0.001$), compared to positive group, also, Cpd1 & Cpd2 groups showed a significant increase to (4.0 ± 0.28 g/dl), (4.1 ± 0.11 g/dl), by 17.6%, 20.5%; respectively ($p < 0.001$), compared to negative group. Also, mean value of Bilirubin level was found to be (0.44 ± 0.0 mg/dl) in negative control group, Positive control group showed a significant increase in Bilirubin level to be (0.56 ± 0.0 mg/dl) by 27.2%, ($p < 0.001$), compared to negative control group. While Cpd1 therapeutic & Cpd2 therapeutic showed a significant decreased to (0.49 ± 0.0 mg/dl), increased (0.57 ± 0.00 mg/dl), by -12.5%, 1.7%; respectively ($p < 0.01$), compared to positive group, also Cpd1 & Cpd2 groups showed a significant increase to (0.55 ± 0.0 mg/dl), (0.55 ± 0.0 mg/dl), by 25%, 25%; respectively ($p < 0.01$), compared to negative group as shown in table (4) and Fig.(18- 21).

Effect of CP1 & CP2 on Kidney functions in all studied groups:

The mean value of Creatinine concentration in serum was decreased from (0.7 ± 0.05 mg/dl) in negative

group to (0.6 ± 0.05 mg/dl) in positive group by 14.3% ($p < 0.001$), compared to negative control group. While concentration was significantly decreased to (0.5 ± 0.05 mg/dl), (0.5 ± 0.05 mg/dl), by -28.5%, -28.5%, in groups of Cpd1 & Cpd2 ($p < 0.01$), compared to negative group. While, their concentration not changed in Cpd1 therapeutic & Cpd2 therapeutic, compared to positive group. While, mean value of Urea concentration was increased from (23 ± 1.15 mg/dl) in negative group to (28 ± 1.15 mg/dl) in positive group by 21.7%, ($p < 0.001$). While, their concentration was significantly decreased to (25 ± 1.15 mg/dl), (26 ± 1.15 mg/dl) by -10.7%, -7.1% in Cpd1 therapeutic & Cpd2 therapeutic ($p < 0.01$) compared positive group, also Cpd1 & Cpd2 groups showed increase to (25 ± 1.15 mg/dl), (24 ± 1.15 mg/dl), by 8.7%, 4.3 %; respectively ($p < 0.01$), compared to negative group. as shown in table (5) and Fig.(21-23).

5. Discussion.

Heterocyclic compounds have a large spectrum of biological activities. Thiazole derivatives are a category of those compounds that have proven considerable antitumor compounds. The synthesized compounds CP1 & CP2 of this study were elucidated their structure by performed IR, HNMR, the theoretical structures were practically approved. In *in vitro* study of the cytotoxicity against human cancer cell lines. The IC₅₀ values of CP1 on HCT116 (colon cancer), MCF-7 (breast cancer) and HEPG2 (liver cancer) found 4.7 ug/ml, 4.8 ug/ml and 11 ug/ml respectively. While IC₅₀ value for CP2 found 9.5 ug/ml, 9.6 ug/ml and 18 ug/ml respectively. The toxicity of studied compounds showed low toxicity various types of cancer cell lines as 1- alkyl- 3- (6- (2- ethoxy- 3- sulfonyl amino pyridin- 5- yl) benzo[d] thiazole- 2- yl) urea was proved to

retain the anti-proliferative activity and the inhibitory activity this is an agreement with **Xie (22)**, who reported a new series of benzothiazole derivatives, with in *vitro* efficacy against HCT116, MCF-7 cell lines. Also, In *vivo* study the LD50 of the studied compounds were safe up to 500 mg/kg and the most effective dose was 15 mg/kg. Thiazole derivatives have no toxic effects on eukaryotes. The volume & count of EAC cells were decreased significantly in both CP1&CP2 treated groups compare to positive control group. This is may be due to the The synthesized compounds showed high binding affinity toward the target p53 protein as tumor suppressor gene by two hydrogen bonds, one hydrogen bond with Glu 285 and another one with LYS 132. So tumor growth was decreased. Also life span of treated groups were elevated ,as thiazole derivatives were consider as a reliable criterion for the depiction of efficacy of an anti-tumor agent **(23)**. This is an agreement with **Perveen (24)** who reported reduction of tumor volume and viable cell count of tumor-bearing mice, tumor cell growth inhibition determined the potency of an anticancer agent. On the other hand thiazole nucleus, heterocyclic derived from thio semi carbazone, the anti-tumor activity of thio semi carbazones seems to be due to an inhibition of DNA synthesis produced by the modification in the reductive conversion of ribo nucleotides to deoxy ribo nucleotides **(25)**. This biological role is often related with their capability to inhibit the enzyme, ribo nucleotide reductase, similar to what is observed with potent anti-cancer drugs such as triapine **(26)**. Hematological parameters, showed decreased HB, RBCS in EAC bearing group and increased in groups treated by thiazole, these results were agreement with **(27) price** which

decrease in RBCs because of iron deficiency, either due to hemolytic or myelo pathic conditions. RBCs count increased in CP1 & CP2 groups by 18.3% and 15.2% respectively compared to EAC control group which supports its reduced myelo toxicity and increased hematopoietic protecting activity and free radical scavenging activity **(28)**. Hepatocytes are damaged in the mice bearing EAC cells causing an increase in the levels of aspartate aminotransferase (AST) and alanine amino transferase (ALT) activity. Therefore, the raise of AST and ALT in the blood is directly related to the extent of the tissue damage **(29)**. CP1&CP2 improve liver function where ALT, Bilirubin were decreased and Albumin was increased. These results are agreement with results of **Huang (30)**. Where the elevation of liver enzymes is an index of impaired liver functions

6. Conclusion: Thiazole derivatives showed anti cancer activities against EAC and human cancer cell lines.

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Docking results Table (1).

Ligand (comp1) (kcal/mol)	Receptor (p53)	Interaction	Distance	E
N 18 (3.4)	GLU 285 (B)	H-donor	3.09	-
O 24	LYS 132 (B)	H-acceptor	3.47	-1.2

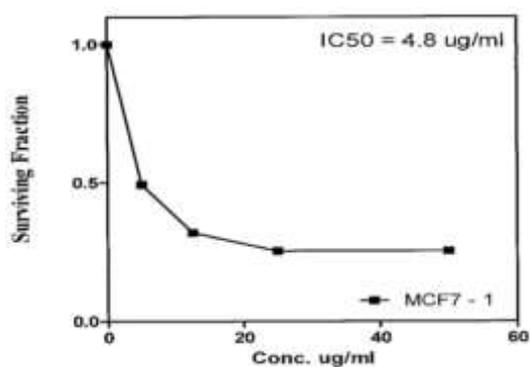


Figure (7): The cytotoxic effect of CP1 on human breast cancer cell line (MCF7).

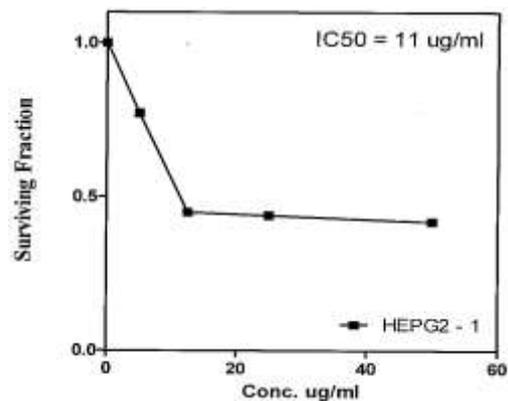


Figure (9): The cytotoxic effect of CP1 on human liver cancer cell (HEPG2).

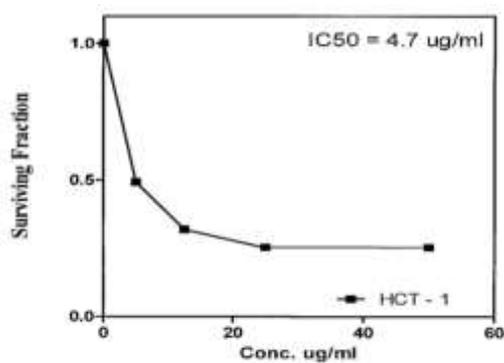


Figure (8): The cytotoxic effect of CP1 on human colon cancer cell line (HCT).

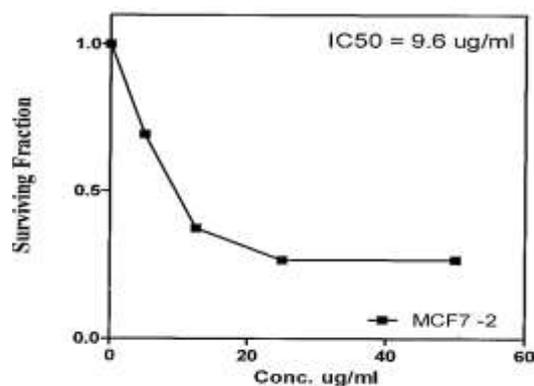


Figure (10): The cytotoxic effect of CP2 on human breast cancer cell. (MCF7)

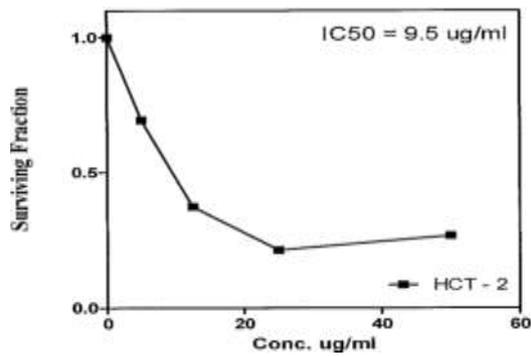


Figure (11): The cytotoxic effect of CP2 on human colon cancer cell (HCT).

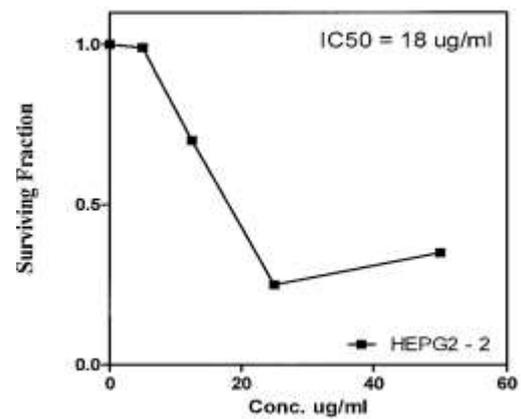


Figure (12): The cytotoxic effect of CP2 on human liver cancer cell (HEPG2).

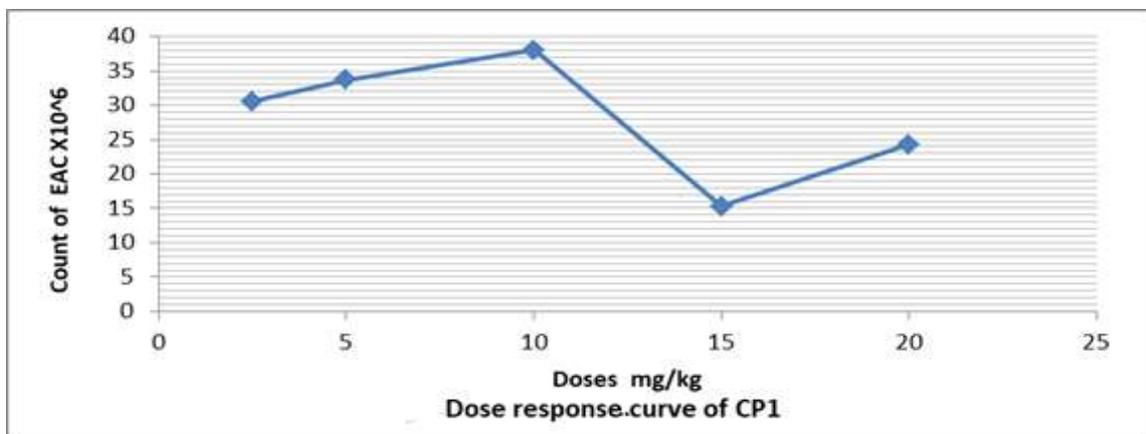


Figure (13): Dose response curve for CP1.

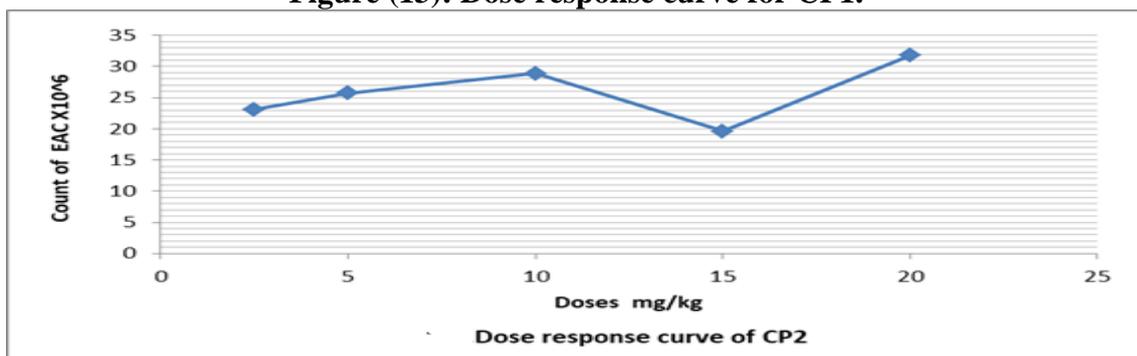


Figure (14): Dose response curve for CP2

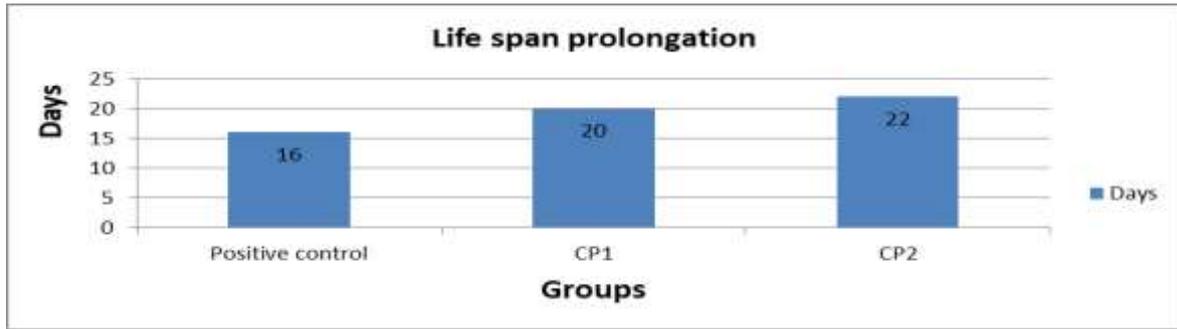


Figure (15): Life span prolongation.

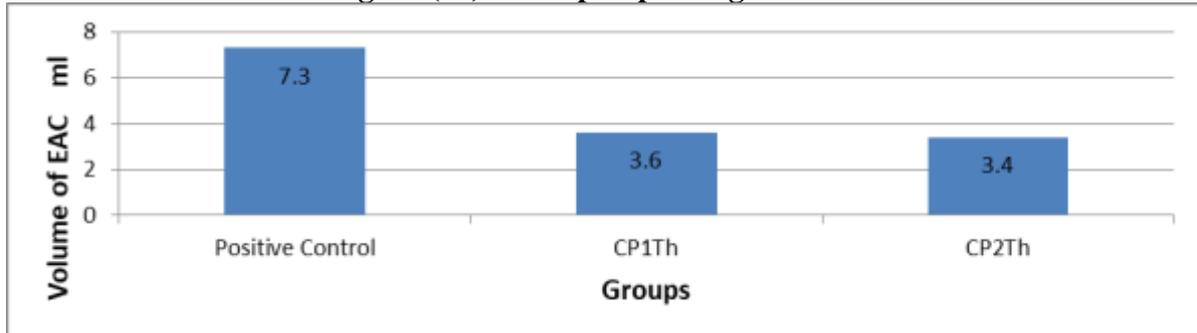


Figure (16): Effect of CP1 & CP2 on volume of EAC.

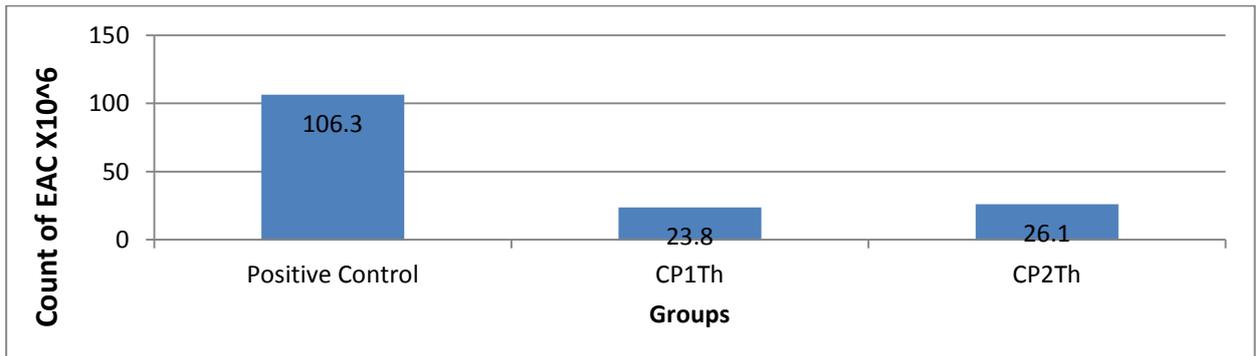


Figure (17): Effect of CP1 & CP2 on count of EAC.

Table (2): Hematological parameters in all studied groups.

Mice Group	Hb g/dL	RBCs (x10 ⁶)	WBCs (x10 ³)	PLT (x10 ³)
	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Negative Control	13.5±0.10	8.75±0.54	8.1±0.08	670±2.88
Positive Control	10.4±0.10**	7.00±0.17*	8.6±0.11*	572±1.15***
Change %	-22.9%	-16.5%	6.2%	-14.6%
Cp1T	14.1±0.010**	8.74±0.31**	19.4±0.05***	683±1.15***
Change %	35.5%	18.3%	125.5%	19.4%
Cp2T	12.4±0.010**	9.2 ±0.14**	11.8±0.11***	706±1.15***
Change %	19.2%	15.2%	37.2%	23.4%

Table (3): Effect of CP1&CP2 on ALT, AST, ALB and Bilirubin .

Groups	ALT	AST	ALBUMIN	BILIRUBIN
	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Negative Control	45±1.15	139±2.88	3.4±0.05	0.44±0.00
Positive Control	62±1.15***	237±1.15***	2.3±0.05***	0.56±0.00
Change %	37.7%	70.5%	-32.3%	27.2%
Cp1	70±2.88**	205±1.15***	4.0±0.28	0.55±0.00
Change %	55.5%	47.4%	17.6%	25%
Cp2	57±1.73**	217±1.73***	4.1±0.11***	0.55±0.00
Change %	26.6 %	56.1%	20.5%	25
Cp1TH	43±1.15***	259±0.57***	3.6±0.05***	0.49±0.00
Change %	55.5%	9.2%	56.5%	-12.5%
Cp2TH	37±1.73***	272±1.73***	3.5±0.05***	0.57±0.00
Change %	-40.3 %	14.7%	52.2%	1.7%

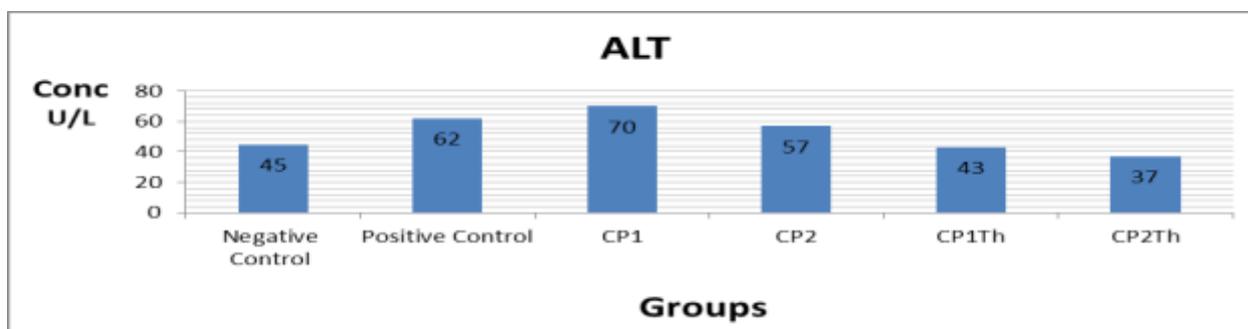


Figure (18): ALT activity in serum in all groups.

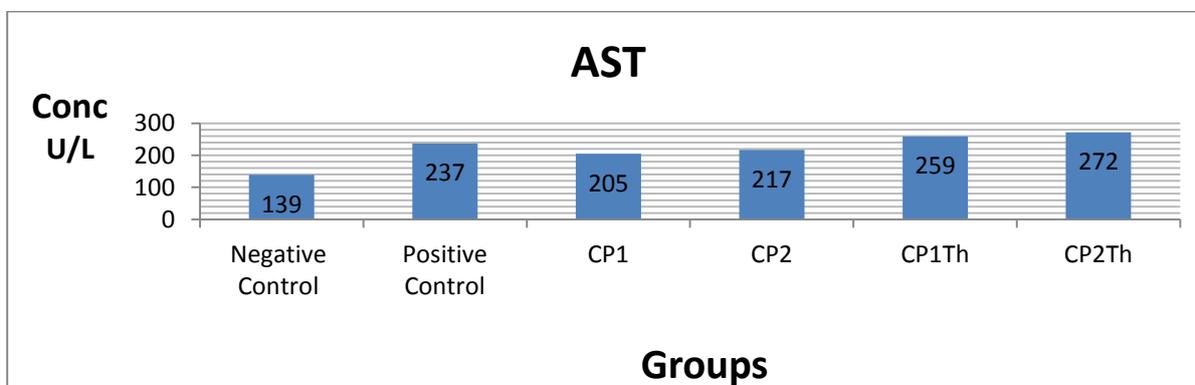


Figure (19): AST activity in serum in all groups.

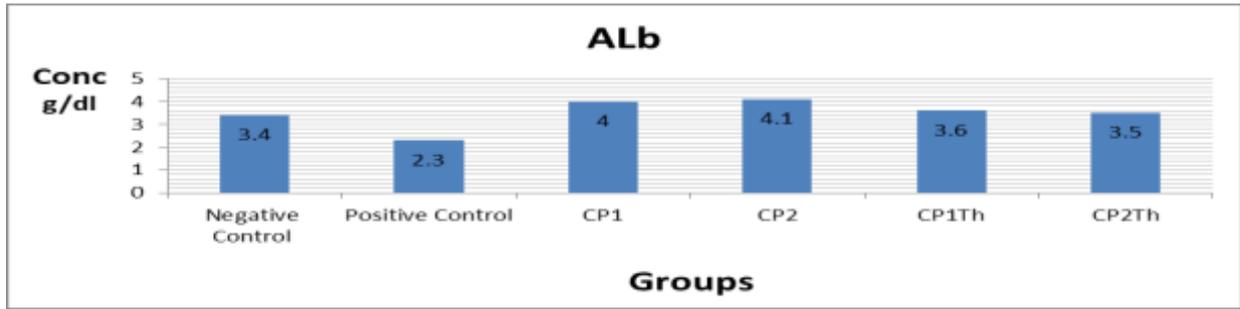


Figure (20): Effect of CP1&CP2 on serum ALB in all studied groups:



Figure (21): Effect of CP1&CP2 on serum T.Bilirubin in all studied groups:

Table (4): Effect of CP1& CP2 on serum Creatinine in all studied groups:

Variiances	Negative Control	Positive Control	Cpd 1	Cpd 2	Cpd 1 Therapeutic	Cpd2 Therapeutic
Mean±SE	0.7±0.05	0.6±0.05	0.5±0.28	0.5±0.11	0.6±0.05	0.6±0.05
%Change	----	-14.3%	-28.5%	-28.5%	-----	-----

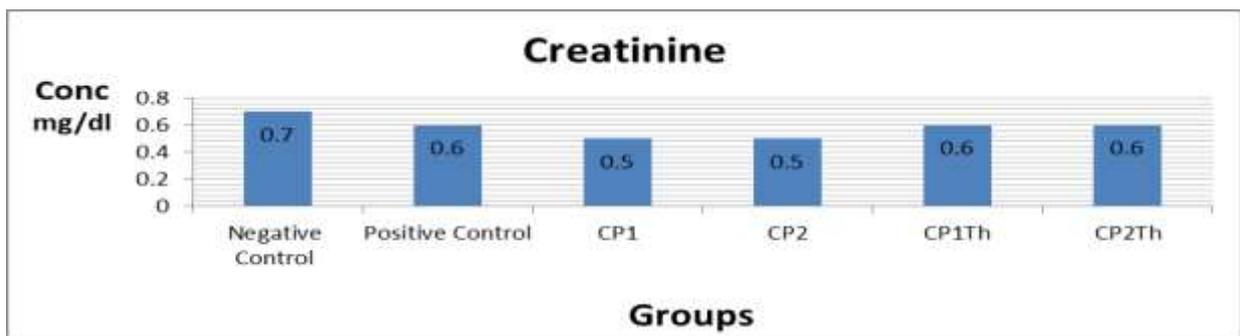
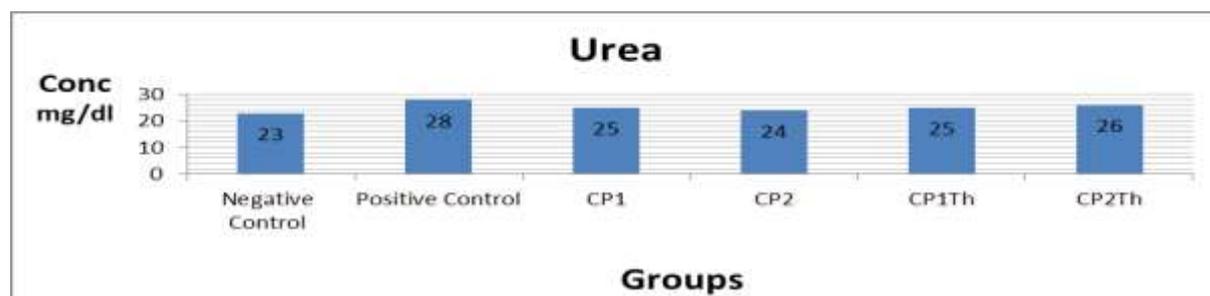


Figure (22): Effect of CP1&CP2 on serum Creatinine in all studied groups:

Table (5): Effect of CP1& CP2 on Urea (mg/dl) in all studied groups.

Variances	Negative Control	Positive Control	Cpd 1	Cpd 2	Cpd 1 Therapeutic	Cpd2 Therapeutic
Mean±SE	23±1.15	28±1.15*	25±1.15	24±1.15	25±1.15	26±1.15
%Change	-----	21.7%	8.7%	4.3%	-10.7%	-7.1%

**Figure (23): Effect of CP1&CP2 on serum Urea in all studied groups**