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Biochemical study in Ehrlich carcinoma cells-bearing mice treated with arsenic trioxide and cisplatin

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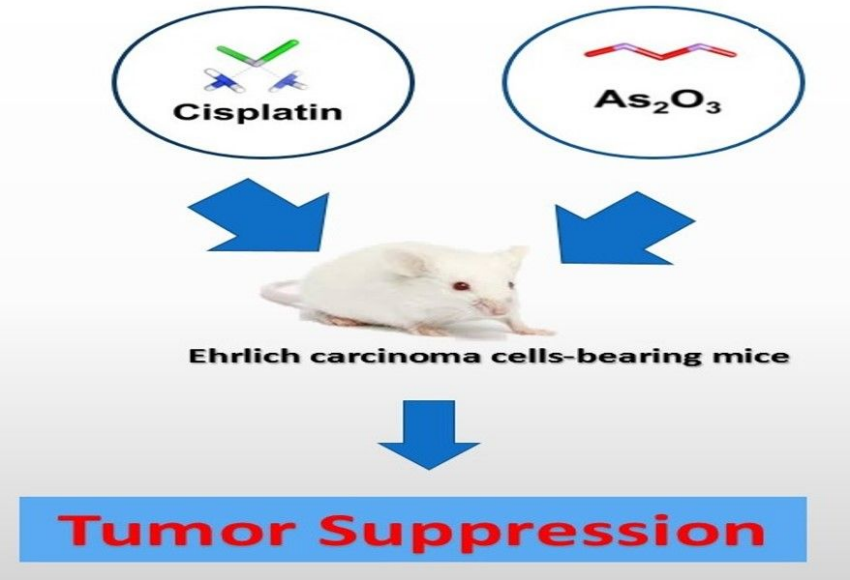
Graphical Abstract



Abstract

The purpose of the study was to evaluate the effect of arsenic trioxide and cisplatin on Ehrlich ascites carcinoma (EAC) bearing mice. Fifty female Albino mice were separated into five groups. Group 1 considered the control. Groups 2 to 5 were injected 2.5 10^6 EAC intraperitoneally (IP). Injected gp. 2 considered the positive control. Groups 3 and 4 received intraperitoneal injections of arsenic trioxide (ATO) (5 mg/kg) and cisplatin (CIS) (2 mg/kg)) daily for 14 days, while Group 5 received a combination of arsenic trioxide (ATO) (5 mg/kg) and cisplatin (CIS) (0.5 mg/kg)) daily for 14 days. After 14 days of treatment, blood samples were collected. Screening of the medications effects used for EAC treatment are carried out by mice body weights, body weight gain, and tumor growth inhibition by counting total, viable and non-viable tumor cells number for each group. Concurrently, liver functions (transaminases, total protein, and albumin) and kidney functions (urea and creatinine) were evaluated to reflect any clinical changes. The study shown that both ATO and CIS have synergetic properties against EAC.

Keywords: Arsenic trioxide, Cisplatin, Ehrlich ascites carcinoma, albino mice

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1. Introduction

Cancer is a multicellular disease that can arise from all types of cells and organs with multiple factors. [1]. Experimental cancer models have been critical in cancer drug discovery because they act as predictors of treatment success or failure [2]. Ehrlich ascites carcinoma is similar to human tumors and is most sensitive to chemotherapy as it is undefined and has a rapid growth rate [3]. Chemotherapy is often regarded as the primary therapeutic option for many types of malignancies, whether with or without surgery [4]. Chemotherapeutic agents used in current clinical practice have significantly reduced mortality/morbidity while also improving patient quality of life [5]. Cisplatin is a highly effective chemotherapy medication used for many types of solid tumors [6]. It is commonly thought that the key biochemical mechanism of cisplatin entails binding the drug to the DNA in the cell nucleus and subsequent interference with normal transcription and/or replication mechanisms [7]. These imbalances may lead to a proliferation that results in cancer cell death. There is much interest in the potential use of arsenic trioxide (ATO) to treat other malignancies such as gastric cancer [8], neuroblastoma [9], esophageal [10], prostate and ovarian carcinomas [11] . The toxicity of ATO in the heart, liver, kidney, and nervous system [12], particularly cardiac toxicity [13], has limited its clinical utilization. Combination treatment is a procedure used in clinical practice to improve the therapeutic impact and lower the toxicity of anticancer medications [14]. Our work was aimed at studying some biochemical and some hematological variations after the treatment of EAC-bearing mice using arsenic trioxide and cisplatin.

2. Materials and methods

2.1. Drugs and reagents

All drugs (ATO & CIS) were obtained from Merck Ltd, (Mumbai, India). The buffer saline used to dissolve the drugs in suitable doses. Kits for biochemical assays were obtained from Biodiagnostics and Research Reagents Co. (Cairo, Egypt).

2.2. Experimental animals

Healthy Female mice with average body weight ranging from 18-22 g were used as experimental animals. The National Cancer Institute (Cairo University, Egypt) was the source of mice. The mice were housed (10 animals per cage) at the animal house at Medical Biochemistry Department, Faculty of Medicine, Tanta University, Egypt. Animals were given typical laboratory food. Water was given with unrestricted access. Mice were allowed to adapt the laboratory environments for one week before starting the experiments. All procedures have been implemented in accordance with Suez Canal University guidelines.

2.3. Ehrlich carcinoma cells

The line of EAC cells was provided from the National Cancer Institute. The tumor line was maintained by serial intraperitoneal implantation of EAC 2.5×10^6 tumor cells/ female mice. The cell viability was assessed using trypan blue assay and counted by haemocytometer before injection into mice for experimentation at a dose of $2.5\text{x}10^6$ EAC cells/mouse [15].

2.4. Experimental design

Fifty female Albino mice were separated into five groups. Gp. 1 considered the control. Gp. 2 to gp. 5 were injected 2.5 10^6 EAC intraperitoneal. Injected gp. 2 considered the positive control. Gp. 3 and gp. 4 were injected IP daily for 14 days with arsenic trioxide and cisplatin with (5 mg/kg) [16] and (2 mg/kg) [17] respectively, and Group 5 was injected IP daily for 14 days with a combination of arsenic trioxide and cisplatin with (ATO 5 mg/kg) and (CIS 0.5 mg/kg) respectively.

2.5. Blood sampling

Blood samples were collected from the retro-orbital venous plexus [18].The blood was divided into two parts. The first part was collected into EDTA tube for hematological examination. The second part was collected in plain tubes then centrifuged to separate serum.

Table 1: Comparison of body weight and body weight gain among the different groups Under study

Groups (n= 10)	Body weights(g) (Mean ± SD)	Body weight gain(g) (Mean ± SD)
1	23.3 ± 0.6	0.1±0.1
2	34.0 ± 0.5***	10.96±1***
3	30.3 ± 1.1***,##	6.2±0.7***,##
4	26.5 ± 1.04*,###	4±0.95*,###
5	25.2 ± 0.4###	2.1±0.5###

(*) denote to major difference in comparison with control group,
(#) denote to major difference in comparison compare with EAC.
* P<0.05 ,** denote to P<0.01 ,*** denote toP<0.001

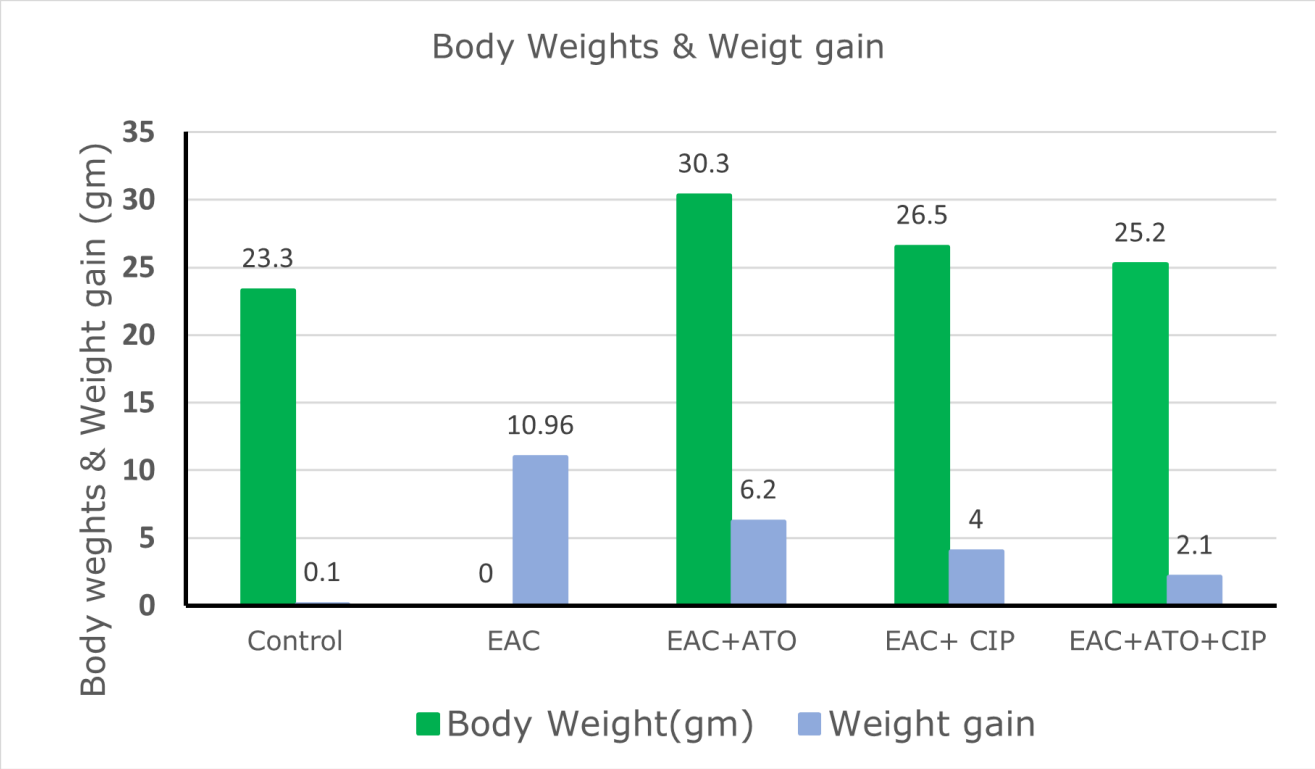

Figure 1: Mice weights & weight gain of differentgroups.

2.6. Assessment of cell viability by trypan blue

After the end of the experiment, the mice were euthanized under the anesthesia, and peritoneal cavity dissected to aspirate EAC aliquot into a test tube. The volume of EAC-fluid for each mouse was measured. The number of viable and non- viable EAC-cells were measured by trypan blue assay. The viable and non- viable EAC-cells were microscopically counted using haemocytometer slide. After mixing of EAC-aliquot with 2% trypan blue, the count of nonstained cells (viable) and stained cells (non -viable) are calculated [19].

2.7. Hematological studies

Valuation of hemoglobin (Hb), Red blood cells, white blood cells, platelets count and differential leukocytic count were accomplished by standard automated counter Mindray BC-2800 Vet analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Shenzhen, China)

Table 2: Comparison of Ascitic volume and Tumor cellcount among the different groups under study

Groups	Ascitic volume ml) (Mean ± SD) Day 14±	Tumor cell count x 106/ml (Mean ± SD)		
		Total	Live	Dead
2	8.60 ± 0.9	115.29 ± 12.54	111.51±12	3.78 ± 1.57
3	5.80 ± 0.33#	20.96 ± 4.5##	15.47 ± 3.8##	5.49 ± 1.29
4	1.57 ± 0.2##	1.71 ± 0.38##	1.14 ± 0.24##	0.57 ± 0.17
5	4.86 ± 0.26##	7.29 ± 0.67##	4.21 ± 0.55##	3.07 ± 0.39

Valuesare expressed as mean ± SD, n=10 for each group.
(#) refer tosignificant difference in comparison compare with EAC
# P<0.05 ## P<0.001

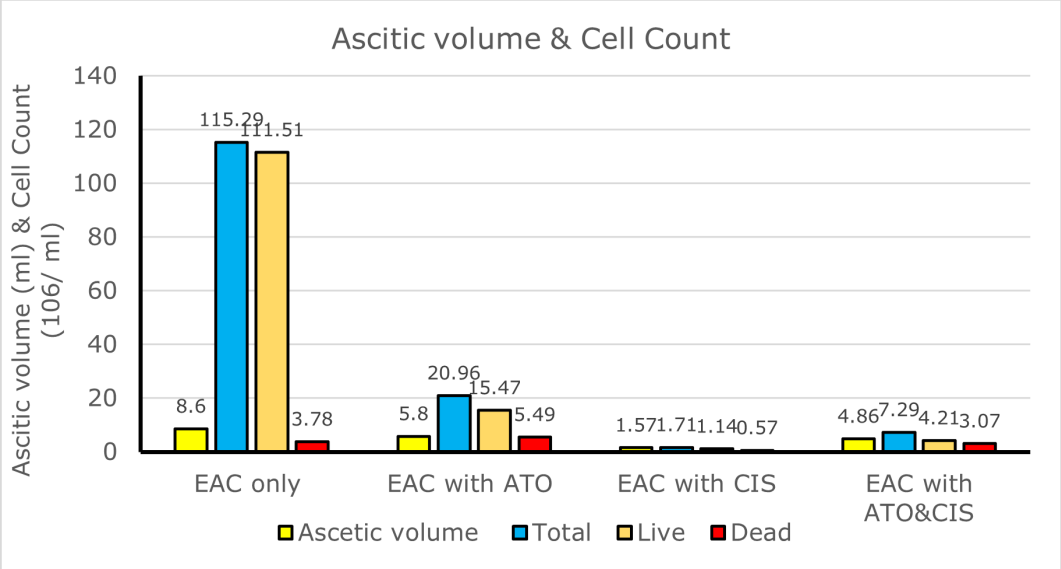

Figure 2: Tumor volume and tumor count of different groups

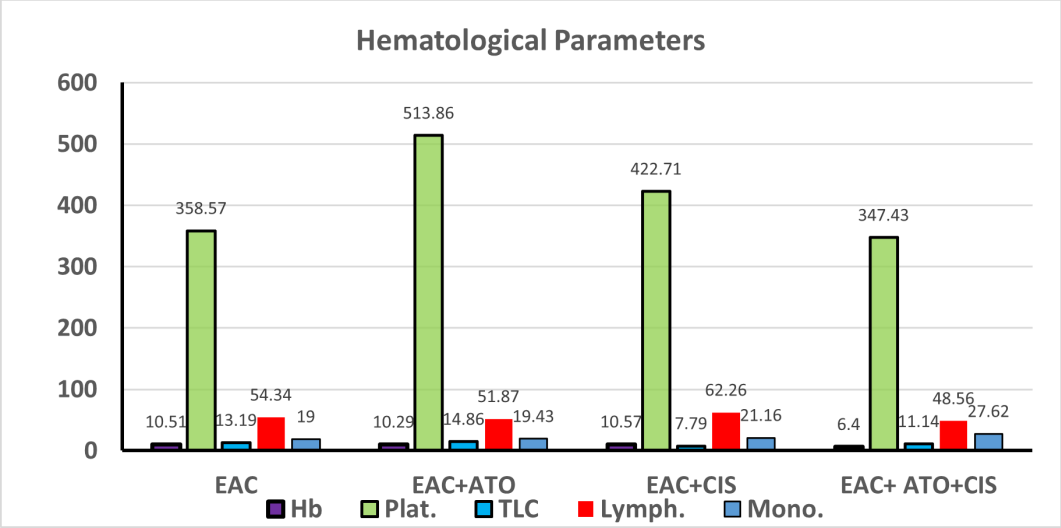

Figure 3: Hematological parameters in different groups

Table 3: Hematologicalparameters in different groups

Groups	Collecting results of CBC (Mean± SD)				
	Hb g /dl	Platelet ×103/μl	WBCs ×103/ μl	Lymph. %	Mono. %
1	11.53 ±0.42	392 ±63.59	7.9 ± 0.95	78.13±0.75	15.54±1.39
2	10.51 ± 0.61	358.57±55.93	13.19±1.59*	54.34±4.84**	19.5±5.29
3	10.29 ± 0.49	513.86±65.35*#	14.86±1.35***	51.87±7.29**	19.43±5.66
4	10.57 ± 0.48	422.71±62.98#	7.79±1.43#	62.26±5.11	21.16±4.82*
5	6.4±1.1***,###	347.43±38.69*	11.14±0.71*	48.56±4.34***	27.62±5.44**

Values represent mean ± SD, n=10 for eachgroup.
(*) denote to major difference in comparison withcontrol group,
(#) denote to major difference in comparison comparewith EAC

Table 4: Comparison of some liver function tests amongthe different groups under study

Groups (n= 10)	ALT (U/L)	AST (U/L)	Albumin (g/dl)	Total protein (g/dl)
1	45.96 ± 2.2	329.07 ± 10	3.07 ± 0.072	5.1 ± 0.1
2	64.63 ± 7.3	481.4 ± 18**	2.53±0.06**	4.6 ± 0.2
3	67.08 ± 4.5*	499.1 ±10.8**	2.6 ±0.09**	4.67 ± 0.32
4	76.06 ± 5.4	508.9 ± 12**	2.73 ± 0.11*	4.61 ± 0.22
5	59.12 ± 7.5	332.8 ± 12##	2.69 ± 0.07*	4.87 ± 0.15

Values are expressed as mean ± CD, n=10 foreach group.
(*) denote to significant difference in comparison withcontrol group,
(#) denote to significant difference in comparisoncompare with EAC

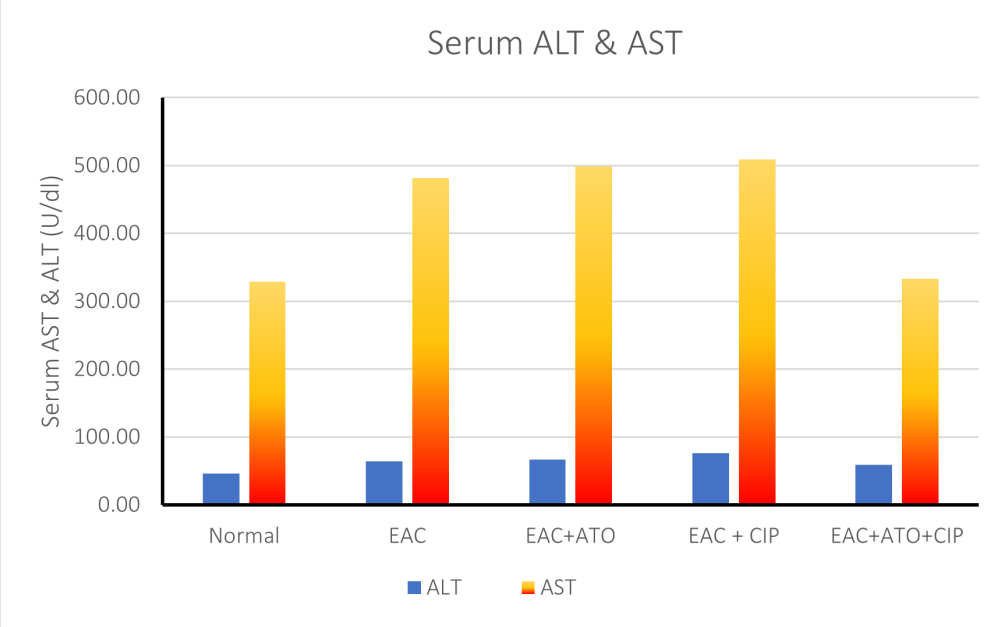

Figure 4: Serum AST & ALT in different groups

Table 5: Comparison of some kidney function tests among the different groups under study

Groups	Urea (mg/dl)	Creat. (mg/dl)
1	$35.05 \pm 0.78^{a,b,c}$	0.57 ± 0.07
2	$45.07 \pm 1.23^{c,d,f}$	0.59 ± 0.062
3	$58.41 \pm 3.89^{a,d,g,j,m,q,r}$	0.61 ± 0.08
4	$48.55 \pm 2.42^{a,g,o,r}$	0.47 ± 0.05
5	$46.71 \pm 1.03^{b,d,q}$	0.57 ± 0.02

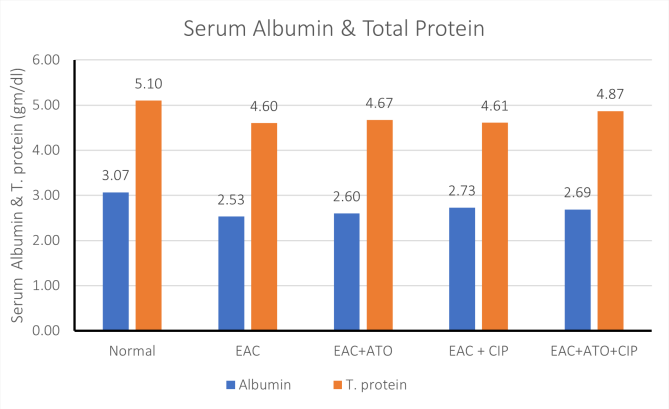

Figure 5: Serum Albumin & T. Protein in differentgroups

2.8. *Biochemical studies*

Serum used for estimation of alanine aminotransferase (ALT) [20] , aspartate aminotransferase (AST) [20], Serum albumin [21], total protein [22], blood urea [23] , and serum creatinine [24].

2.9. *Statistical analysis*

SPSS ver. 22 was used for Data analysis. Normally distributed data were plotted as mean ± standard deviation. One-way ANOVA used for differences between groups [25].

3. Results and discussion

Current work has shown that the increased body weight of untreated EAC mice (group 2) is significantly higher than other groups due to the rapid and gradual accumulation of tumor cells. Treatment of EAC mice with ATO, CIS and the combination of ATO and CIS has significantly reduced body weight gain when compared to untreated EAC mice due to reduced tumor size (Table 1). The present study showed that administration of ATO and /or CIS showed a significant reduction of the tumor volume, tumor count (total and viable) and increased the percentage of trypan blue positive stained dead cells in EAC -bearing-treated mice with best results in co-administrated group (Table 2).

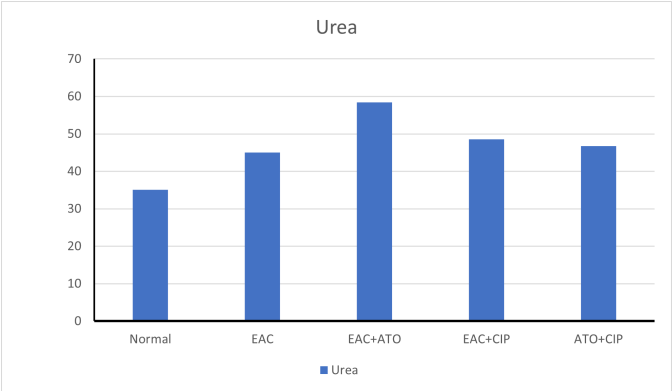

Figure 6: Serum Urea in different groups

3.1. *Effect of treatment of ATO and/or CIS on some hematological parameters in different mice groups.*

Hemoglobin (Hb) showed significant decrease in treated group compared with normal control (group 1) this decreasing may due oppressive effect of EAC on the erythropoiesis [26]. A significant increase in platelet of ATO -treated group compared to negative and positive control groups. Platelet showed a decrease in CIS- treated group and ATO + CIS treated group compared to control group. Platelet count reduced in gp.2 this reduction may be due to bone marrow suppression [26]. White blood cells (WBCs) showed a significant increase in EAC bearing positive control group (group 2) and ATO treated group (group 3) compared to normal control group. Propagation of EAC cells may be attributed to this increase due to inflammatory reaction or stress [27]. Lymphopenia in all groups compared to negative control may attributed to drug immunosuppressive [28] .

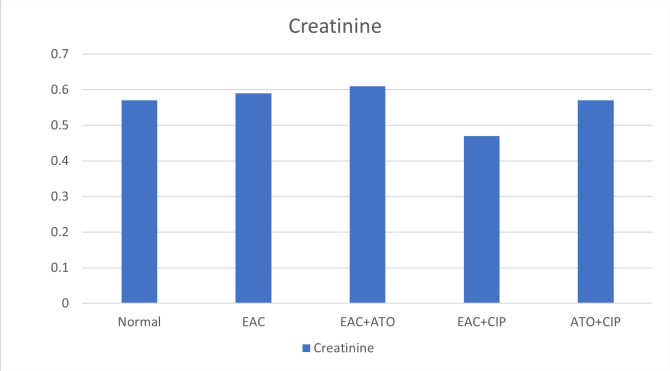

Figure 7: Serum Creatinine in different groups

3.2. Effect of treatment of ATO and/or CIS on biochemical parameters in different mice groups

In the current study serum ALT and AST activity showed major increase in EAC-bearing (gp. 2) mice compared with the normal group (gp. 1) because inoculation of Ehrlich cells induce organ dysfunction and metabolic disturbance [29]. Hepatotoxic effect of arsenic mainly the cause of increment of ALT and AST activities through the leakage of these enzymes into the blood stream [30]. The abnormalities in albumin levels indicate liver dysfunction. Serum albumin was significantly decreased in EAC bearing positive control group (gp. 2) and CIS treated group (gp. 4) in comparison with normal group. Hence, the results explained the deleterious effect of CIS on liver cells. Concomitantly, there is no significant change in ATO/CIS treated group denoting the improvement in the therapeutic effect and reduction of the anticancer drugs toxicity. Serum total protein concentration showed no significant rise in treated mice [31] . Blood Urea concentration (mg/dL) was significantly increased in EAC bearing mice (gp. 2), ATO treated group (gp. 3), CIS treated group (gp. 4) and combination ATO plus CIS treated group (gp. 5) when compared to normal control group due to ATO nephritis.

4. Conclusions

The results of the study revealed that combining ATO with CIS provides synergistic effects against EAC that are preferable to either one alone. As a result of their synergetic effect on cancer, we advocated combining ATO with CIS treatment.

5. Ethical issues

Our experiments were carried out in compliance with the guidelines of Animals Ethics Committee.

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