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## Alleviation of Cisplatin-induced Toxicities by Encapsulation Into Liposomes in the Absence or Presence of Ginkgo Biloba.

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**Abstract:** Over the last three decades, cisplatin has been one of the most effective cytotoxic agents, but its administration has been hindered by its nephrotoxicity, neurotoxicity, myelotoxicity, and retinal toxicity. Recently, liposomal cisplatin has been formulated and tested thoroughly in preclinical studies (in vitro). Experiments on animals showed that liposomal cisplatin is less toxic than cisplatin while keeping its anti tumor function. Antioxidants are intimately involved in the prevention of cellular damage by means of reducing the adverse effects of reactive oxygen and reactive nitrogen species on the biochemical processes in the body, hence, they play an important role in the protection and defense mechanisms of the body. This study aims to reduce the adverse effects of cisplatin on the blood and the electroretinogram (ERG) of rabbits through the administration of liposomal cisplatin and by liposomal cisplatin combined with Ginkgo biloba (G.b). To achieve this goal, 36 albino rabbits were used and were divided into 6 groups, the first group didn't receive any treatment (control group), the second was given Ginkgo biloba only, the third one was administrated with IP cisplatin only, and the fourth was given oral Ginkgo biloba plus IP cisplatin. The fifth and sixth groups were administrated with liposomal cisplatin and liposomal cisplatin plus Ginkgo biloba respectively. Our results indicate that there was a reduction of some hematological parameters and ERG waves after the administration of cisplatin, while these values reached comparable values to the control level in the fifth and sixth groups.

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**KeyWords:** Cisplatin, Liposomal cisplatin, Ginkgo biloba, ERG, Ocular toxicity, Chemotherapy, DSC, Haematotoxicity, Liposomes.

### Introduction

Cisplatin is one of the most remarkable successes in 'the war on cancer'. Since the accidental discovery four decades ago, cisplatin has been widely used for chemotherapy. It has shown activity against a broad spectrum of tumors, including testicular, ovarian, cervical, bladder and lung cancers as well as solid tumors resistant to other treatment regimens (Kart, 2010; Yousef, 2009). Cisplatin is potent, demonstrating one of the highest cure rates, for example, over 90% in testicular cancers (Wang and Lippard, 2005). The cytotoxic effect of cisplatin is believed to result mainly from its interaction with DNA, via the formation of covalent adducts between certain DNA bases and the platinum compound (Yang, 2006).

Unfortunately, cisplatin treatment is plagued by problems - including resistance, both intrinsic and acquired, and its strong side effects (Kartalou and Essigmann, 2001). These include nausea, vomiting, myelosuppression, sensitivity reactions, nephrotoxicity,

ototoxicity, neurotoxicity, gastrointestinal toxicity and bone marrow suppression.

The optic nerve and retina are common sites for chemotherapeutic complications. In some patients treated systemically, cisplatin has produced optic neuritis, papilledema and retinal toxicity that manifests as color blindness (Ostrow, 1978). Intracarotid infusion can lead to visual loss from severe retinal and/or optic nerve ischemia, pigmentary retinopathy or exudative retinal detachment (Margo and Murtagh, 1993).

Cisplatin can damage tissues and cells in the bone marrow. This can result in infections, anemia, or low platelet count leading to easy bruising. Moreover, Decreased hemoglobin levels and leucocyte counts were observed with cisplatin administration (Bogin, 1994). Electrolytes disturbances are also associated with cisplatin therapy (Blachley and Hill, 1981).

It was also shown that cisplatin-based chemotherapy induces a fall in patient plasma concentrations of various antioxidants (Weijl, 1998). This may lead to

failure of the anti-oxidative defense mechanism against free radical-mediated organ damage and genotoxicity of cisplatin that may lead to the induction of secondary malignancies in normal tissues (Wozniak, 2004).

The leaves of Ginkgobiloba tree have been used in traditional Chinese medicine for several hundreds of years and the extract obtained from the leaves has been introduced into Western medicine due to its therapeutic actions in nervous and circulatory systems. EGb761, a standardized extract of Ginkgobiloba leaves, is reported to alleviate symptoms or has neuroprotective effects in various central nervous system disorders such as dementia, cerebral insufficiency, ischemia, traumatic brain injuries, retinal degeneration, vestibular dysfunction, and anxiety. The extract also enhanced mental and cognitive functions in experimental animals or human subjects (for a review, see MacLennan 2002). Ginkgobiloba was found to be effective in preventing some functional and morphological deteriorations in cisplatin-induced peripheral neuropathy (Ozturk, 2004).

Applications of liposomes as drug carriers are becoming apparent. In particular, acute and chronic toxicities associated with certain drugs can be reduced if the agent is presented in association with liposomes. This reduced toxicity is accompanied by maintained or enhanced efficacy. Therefore, the liposomal carrier can provide a significant improvement in the therapeutic index of the entrapped drug.

Distearylphosphatidylcholine (DSPC) vesicles are used as model membranes since this phospholipid is able to mimic many aspects of biological membranes, being one of their most abundant constituents.

The present study was undertaken to evaluate the chemoprotective efficacy of liposomal cisplatin combined with Ginkgobiloba against cisplatin-induced toxicities in albino rabbits.

## Materials And Methods

### Chemicals

High Purity Distearylphosphatidylcholine (DSPC), with a molecular weight of 790.145 (99% pure) was purchased from Lipoid KG (Ludwigshafen, Germany), Trizma buffer, with a molecular weight of 121.1 was purchased from (Sigma chemicals, Steinheim, Germany). Ethanol was of analytical grade and obtained from Merck (Heliopolis, Cairo, Egypt). Cisplatin (50 mg/50ml) with a molecular weight of 300.05 was purchased from (Laboratoire Roger Bellon, France). Ginkgobiloba powder with a molecular weight of 1689.2 from EIPICO (Egyptian International Pharmaceutical Industries Co, Egypt) was used. All other chemicals used in this work were of research grade. Solutions were prepared in de-ionized ultra pure water.

### Liposome Preparation

A molar ratio 7:2 of Distearylphosphatidylcholine (DSPC) to Cisplatin was used to prepare neutral multilamellar vesicles (MLVs) using the method of (Bangham, 1974). DSPC was first thoroughly dissolved in ethanol to assure homogeneity, then, the organic solvent was removed by rotary evaporation yielding a thin lipid film on the sides of a round bottom flask. The lipid film is thoroughly dried to remove residual organic solvent by placing flask on a vacuum pump. Hydration of the dry lipid film is accomplished simply by adding tris-buffered saline (10 mM Tris and 140 mM NaCl at pH 7.4) to the container of the dry lipid and agitating at a temperature higher than the phase transition temperature of the lipid.

Multilamellar vesicles (MLV) are formed of final lipid concentration of (10 mg/ml). For the DSPC/Cisplatin liposomal sample, hydration of the dry lipid thin film by adding of 5 ml of tris buffer containing cisplatin. The flask was mechanically shaken for 15 min at 45°C. The suspension was then centrifuged three times at 11000 rpm for 15 min to remove non-encapsulated drug. Multilamellar liposomes containing Ginkgobiloba were prepared from a lipid mixture of DSPC and Ginkgobiloba in a molar ratio of 7:2. The mixture was taken to 50 ml round bottom flask. Then 20 ml of ethanol was added, and the flask was shaken until all lipids dissolved in the ethanol. The solvent was evaporated under vacuum using rotary evaporator until a thin dry film of lipid was formed. The flask was then left under vacuum for 12 hrs to ensure the evaporation of all traces of ethanol. 5 ml of tris buffer containing cisplatin was then added to the flask which was flushed through with nitrogen stream and immediately stoppered. The flask was mechanically shaken for 15 min at 45°C. The suspension was then centrifuged three times at 11000 rpm for 15 min to remove the non-encapsulated drug.

### Encapsulation Efficiency Measurements

The encapsulation efficiency of the samples was measured using spectrophotometer (Uvikon 930, Italy). The wavelengths were adjusted at 305 nm (the resonance absorption peaks of cisplatin). The absorption of the supernatant of each sample (centrifuged at 8000 rpm for 20 minutes) was compared to the standard curve relating absorption to cisplatin concentration. Then encapsulation efficiency of liposomal cisplatin was calculated from the formula:

Encapsulation efficiency = (total amount of the drug - amount of the free drug) / total drug) x 100.

### DSC Measurements

Differential scanning calorimetry (DSC) is used to measure the melting point ( $T_m$ ) of a sample or the gel/fluid transition temperature in liposomes. Fluidity

of lipid bilayers depends on the type of the lipids or their combination used, and their fluid gel transition temperature (Rudra, 2010). The gel state (ordered) to fluidity (disordered) of lipid was observed by sensitive calorimetric instrument.  $T_m$  represents the peak temperature of the endotherm for the lipid gel-to-fluid phase transition recorded during the heating scan. The pre and main transitions are often observed in phosphatidylcholine (PC) lipids. The pre-transition, which corresponds to the conversion of a lamellar gel phase to a rippled gel phase, is mainly related to the polar region of phospholipids; while the main transition reflects the change from a gel phase to a liquid crystal phase. The experiments were performed with the differential scanning calorimeter (model TA-50 WSI, Schimadzu) calibrated with *Indium*. Samples of empty and drug-loaded multilamellar liposomes were submitted to DSC analysis. The analyses were performed on 5mg samples sealed in standard aluminum pans. Thermograms were obtained at a scanning rate of 5°C/min. Isotonic PBS buffer (pH 7.4) was employed as reference. Each sample was scanned between 0 and 200°C. The temperature of maximal excess heat capacity was defined as the phase transition temperature.

#### **Animals and Experimental Design:**

Thirty six, male, New Zealand, albino rabbits weighing 1.5-2 Kg were used in the study. They were housed individually in separate cages under veterinary supervision. They were used in accordance with institutional guidelines and with the statement for use of animals in ophthalmic and vision research. The rabbits were fed with the standard diet and water ad libitum and kept in a 12 hours dark/light cycles under controlled temperature and humidity. The animals were randomly divided into six groups. Each group consisted of six rabbits and received the following treatment:

Group I: Control group remained untreated.

Group II: received Ginkgobiloba orally (1mg/kg).

Group III: received cisplatin IP injection of (1mg/kg).

Group IV: received Ginkgobiloba orally (1mg/kg), followed by administration of cisplatin IP injection of (1mg/kg).

Group V: received liposomal cisplatin IP injection of (1mg/kg body).

Group VI: received liposomal cisplatin combined with Ginkgobiloba IP injection of (1mg/kg).

#### **Experimental Protocol**

Every group of rabbits was given the type of treatment twice a week for fifteen days. The measurements were taken after 3, 10 and 15 days.

#### **1-Determination of Hematological Parameters**

For the evaluation of various blood parameters, blood was collected from the auricular vein into a sterilized tube containing heparin and the testswere made on the same day. The collected blood was mixed gently. Plasma was separated from the blood after being centrifuged at 3000 rpm for 20 min in disposable *Eppendorff* tubes and stored at -70°C till analysis. Red blood cells (RBCs) and hemoglobin (Hb) content were analyzed following the criteria of *Dacie and Lewis (1975)*. White blood cells (WBCs) count was done following the method of *Swarup, 1981*.

Finally, the different blood cations, such as calcium; potassium; sodium and magnesium, were estimated in the previously prepared plasma according to *Kingsley and Schaffert 1954; Sidney (1955)*.

#### **2-Electrophysiological Study**

The rabbits were dark adapted for 2 hours before the electrophysiological recording and anesthetized by intramuscular injection. The ERG was recorded by using three Ag -Ag CLskin electrodes. The active electrode was placed near the margin of the lower eyelid, the reference electrode was placed on the forehead and the earth electrode was clipped to the earlobe. Fifty flashes were used with a flash energy of 0.2 joule and a flash frequency of 1 Hz i.e. one flash per second and background intensity of zero. The flashes were derived from the used system which is a computerized system (*EREV 99 apparatus, lace Elettronica Co-, Italy*).

#### **Statistical Analysis**

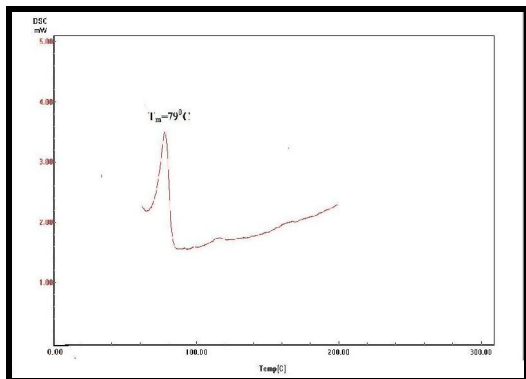
Statistical comparison was performed between treated and untreated eyes using the Student's t- test. The results were presented as the mean  $\pm$  SD and studies were repeated at least four times independently. Differences were considered significant at  $P < 0.05$ .

#### **Results**

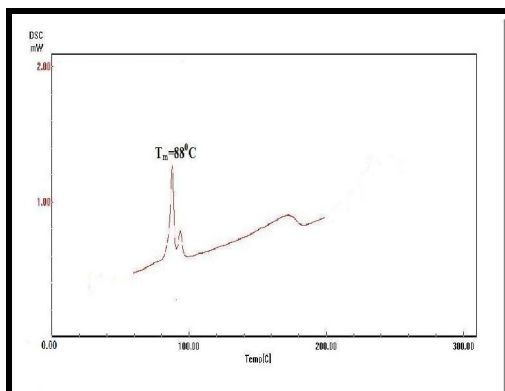
##### **DSC Study**

When submitted to DSC analysis, pure DSPC vesicles upon dehydration showed a major endothermic peak at 79°C (Fig.1), in accordance with *Koynova and Caffrey (1998)*. The increase in phase transition temperature was caused by the decrease in the spacing between the head groups, which allowed for increased van der Waals interactions between the lipid hydrocarbon chains (*Crowe, 1998*).

The incorporation of cisplatin into DSPC liposomes resulted in a shift of the major characteristic endothermic peak of pure DSPC from 79°C to a sharp endothermic peak at 88°C (Figure 2).



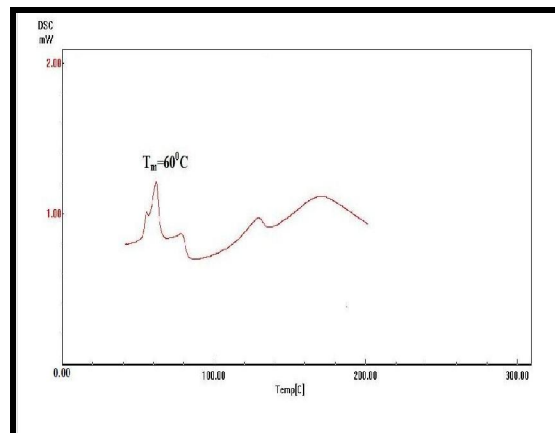
**Figure (1): Calorimetric Scans of Lyophilized Pure DSPC Liposome.**



**Figure (2): Calorimetric Scans of Lyophilized DSPC Liposome Combined With Cisplatin.**

Figure 3 represents the calorimetric scans of vesicles of DSPC combined with cisplatin in the presence of Ginkgobiloba. The presented scans show that the addition of Ginkgobiloba caused a distinct broadening of the main endothermic peak. Here one can observe

the greater effect of Ginkgobiloba upon the incorporation into liposomal cisplatin in comparison to the pure DSPC and DSPC combined with cisplatin alone. The main transition temperature of DSPC liposomes (79°C) is strongly shifted to lower temperature (60°C) for DSPC liposomes encapsulated cisplatin in the presence of Ginkgobiloba.

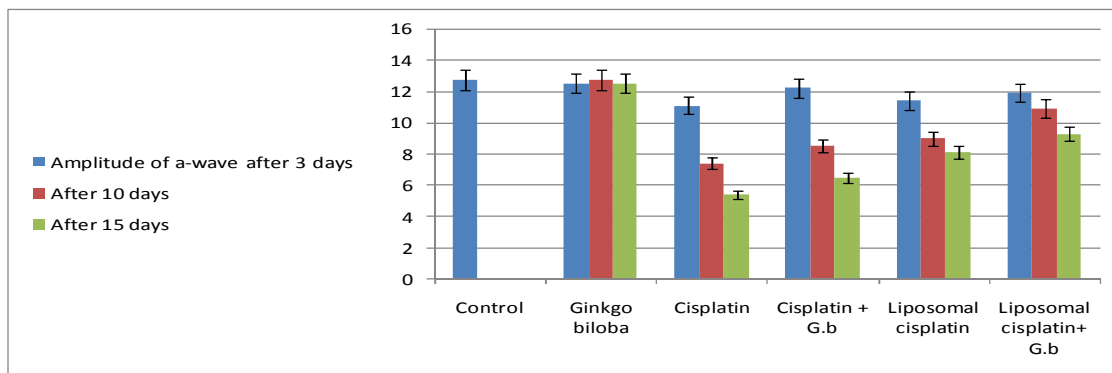


**Figure (3): Calorimetric Scans of Lyophilized DSPC Liposome Combined with Cisplatin in The Presence of Ginkgo Biloba.**

**Electrophysiological Study**

The ERG responses of the dark adapted eyes for the six groups were illustrated in figures 4 and 5 and their characteristic values are given in table 1.

The obtained ERG showed no significant differences in the second group which was administered with Ginkgobiloba only ( $p > 0.05$ ) during the whole period of experiment. Also there were no significant changes in all groups after three days from the administration in comparison with control group.



**Figure (4): ERG (a-wave) Amplitudes of Selected Rabbit Eyes for All Groups.**

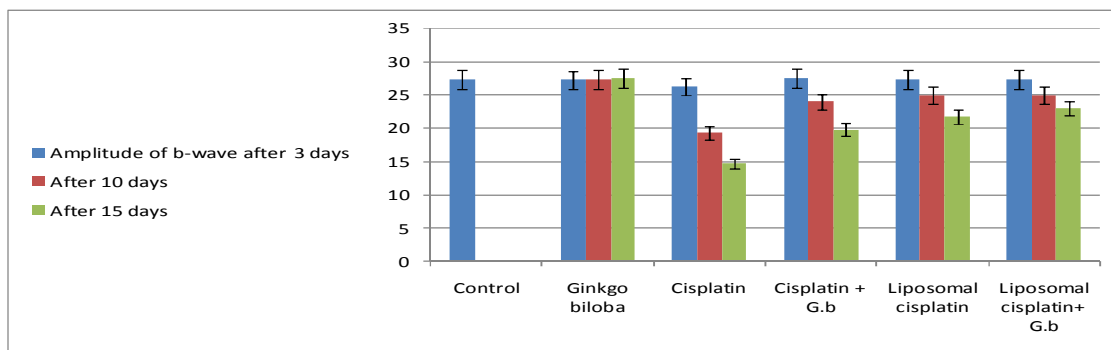


Figure (5): ERG (B-Wave) Amplitudes of Selected Rabbit Eyes for All Groups.

Table 1: Mean Values (±SD) of a- and b- Waves Amplitudes (µV) and Their Implicit Time (mSec) after 3, 10, and 15 Days for all groups.

Groups	Amp of a(µV)			Implicit time of a-wave(mSec)			Amp of b(µV)			Implicit time of b-wave(mSec)		
	3	10	15	3	10	15	3	10	15	3	10	15
Control	12.7±0.5			22.5±1.1			27.3±1.2			46.4±2.1		
Ginkgo biloba	12.5±0.4	12.7±0.4	12.5±0.6	22.5±0.9	22.4±1.1	22.3±0.9	27.2±1.4	27.3±1.1	27.5±0.81	53.4±1.5	53.6±1.3	53.4±1.5
Cisplatin	11.1±0.6	7.4±0.2	5.4±0.4	26.7±0.9	35.2±0.9	40.5±1	26.2±1.1	19.3±0.7	14.74±0.6	46.4±1.1	53.4±0.9	61.5±0.8
Cisplatin + G.b	12.2±0.31	8.5±0.6	6.5±0.41	22.9±0.81	30.1±0.5	33.7±0.34	27.5±0.6	24±0.5	19.8±0.6	46.4±0.8	50±0.7	56.2±0.9
Liposomal cisplatin	11.4±0.41	9.6±0.51	8.1±0.5	22.5±0.74	26±0.51	33.1±0.7	27.3±0.6	25±0.42	21.7±0.5	47.8±1.1	52.8±0.9	53.5±0.81
Liposomal cisplatin + G.b	11.9±0.5	10.9±0.5	9.3±0.4	22.1±0.4	23.3±0.61	27±0.45	27.3±0.6	25±0.41	23±0.5	46.1±0.9	50.1±0.72	50.15±0.6

It is noticed that there was an obvious effect in the third group which was administrated with cisplatin only in the form of reduction in a- and b- wave amplitudes and increase in their implicit time (figures 6 and 7) in comparison with control group along the period of experiment.

The percentage difference was 57.5% and 46% for a- and b- wave amplitudes and -08% and -32.5% for their implicit time respectively, i.e. a-wave is more affected than b-wave. After the administration of

cisplatin with Ginkgobiloba, it is noticed that there was some sort of improvement in ERG parameters and this improvement continues during the period of experiment but still lower than the control eyes as shown in figures 4-7. The percentage difference for the amplitude of the (a) and (b) waves was 48.8 % and 27.5 % respectively. The improvement in ERG parameters was better when using encapsulated cisplatin (the fifth group,  $p < 0-005$ ).

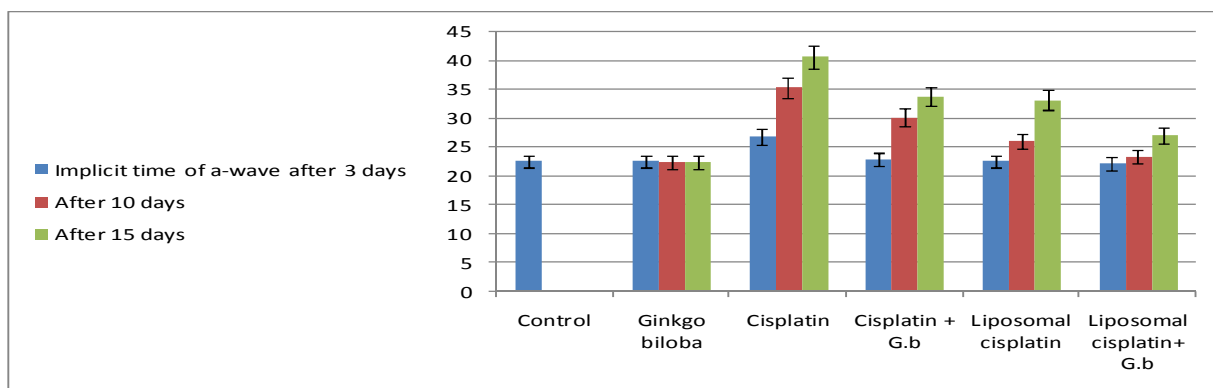
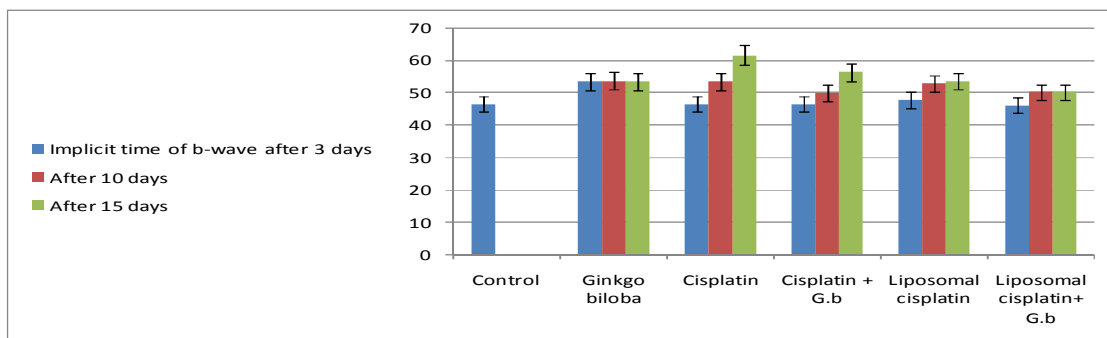


Figure (6): Implicit Time of a-Wave of Selected Rabbit Eyes for All Groups.

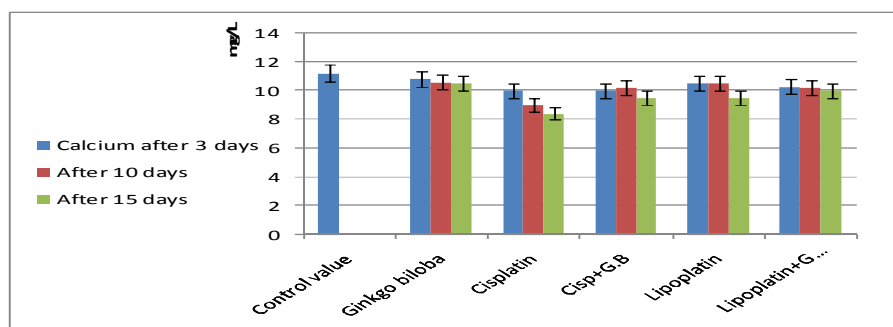


**Figure (7): Implicit Time of b-wave of Selected Rabbit Eyes for All Groups.**

The significant improvement was found in the sixth group which was administrated with encapsulated cisplatin and Ginkgobiloba. This administration maintained ERG parameters near to normal values. The percentage difference of the amplitude of both the a-wave and b-wave was 16.5 % and 16.8 %, respectively ( $P < 0.01$ ) and for their implicit time, the percentage difference was -21.3% and -8.15% for the a-and b-wave respectively ( $P < 0.001$ ).

As compared to the control, no remarkable changes in the group which was administrated with Ginkgo-bilboa only. All plasma cations are presented in the table (2). It is noticed that, there is a slight decrease ( $P < 0.01$ ) in the total calcium content in the group which treated with cisplatin only from the third day after administration of dose (1mg/kg). The decrease was more pronounced ( $P < 0.001$ ) as the administered dose of cisplatin increased to 5mg/kg (25%) by the 15th day of treatment (figure 8).

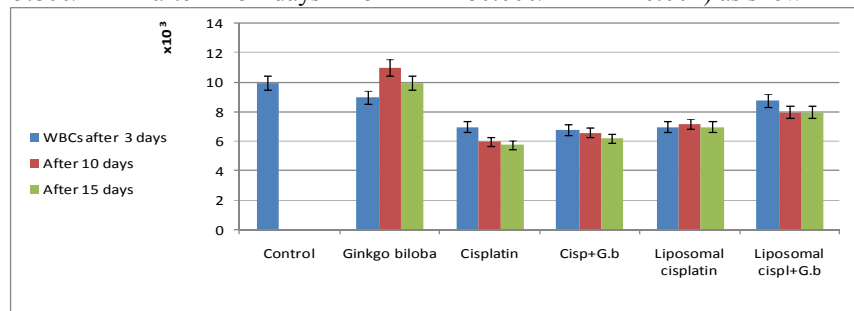
#### Blood Results



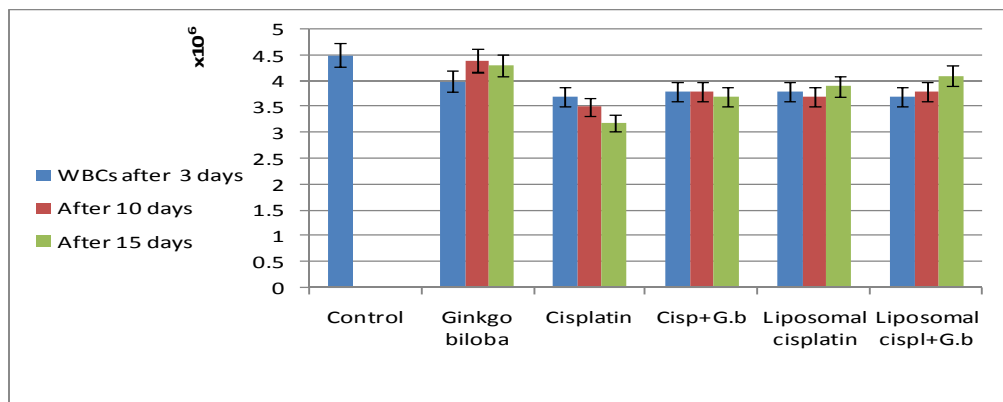
**Figure 8: Plasma calcium level for selected rabbits of all groups after 3, 10, and 15 days.**

No significant changes were observed in the rest of measured cations (Magnesium, sodium and potassium), which remained in the control level in the 3rd group. Also the administration of cisplatin only resulted in decrease ( $P < 0.01$ ) in the white blood cells (WBCs) from 10.000 to 5.800/mm<sup>3</sup> after 15 days from

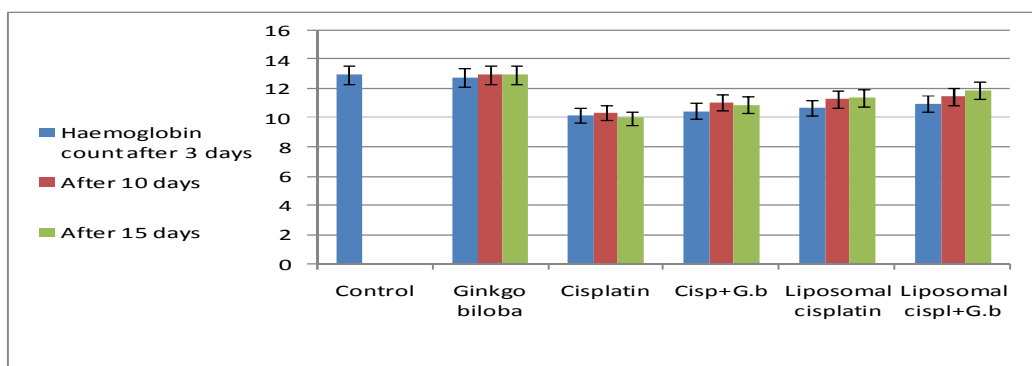
administration (figure 9), moreover, the red blood cells (RBCs) decrease from 4.5 to 3.4 mil/mm<sup>3</sup> ( $P < 0.01$ ) (figure 10), also hemoglobin (Hb) was decreased (figure 11) from 13 mil/mm<sup>3</sup> to 10 mil/mm<sup>3</sup> ( $P < 0.01$ ) and the platelets decreased (figure 12) from 250.000 to 180.000/mm<sup>3</sup> ( $P < 0.001$ ) as shown in table (3).



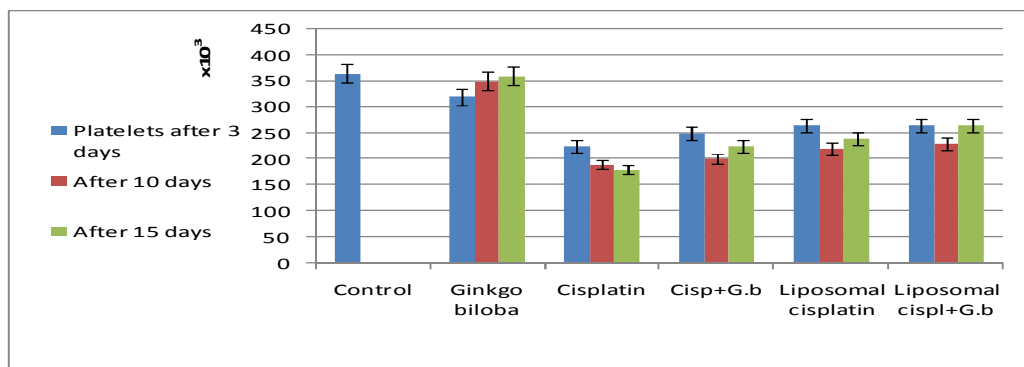
**Figure (9): WBCs Count for Selected Rabbits of All Groups after 3, 10, and 15 Days**



**Figure 10: RBCs Count for Selected Rabbits of All Groups after 3, 10, and 15 Days**



**Figure 11: Hemoglobin Count for Selected Rabbits of All Groups after 3, 10, and 15 Days**



**Figure 12: Platelets Count for Selected Rabbits of All Groups after 3, 10, and 15 Days**

As compared to cisplatin alone, the combined administration of rabbits with oral Ginkgo-biloba plus intrapretoneal cisplatin resulted in an improvement in the total calcium which reached 9.5 mg/dl, WBCs count was 6.200/mm<sup>3</sup>, RBC count was 3.7 mil/mm<sup>3</sup>, hemoglobin was 10.9 and finally the platelets were 225.000/mm<sup>3</sup>. The all above hematological parameters improved after the administration of encapsulated cisplatin (liposomal cisplatin), the calcium content reached 9.5 mg/dl and WBC count

was 8.800/mm<sup>3</sup>, RBCs count was 3.9 mil/mm<sup>3</sup>, Hb was 11.4 and platelets were 290.000/mm<sup>3</sup>. Further improvement appeared in the 6<sup>th</sup> group which was administered with liposomal cisplatin combined with Ginkgo-biloba. This improvement is higher than the 3<sup>rd</sup> group and near to the control level; where the percentage difference in calcium was 1.7%, in WBCs was 5%, in RBCs was 8.9%, in Hb was 8.46% and in the platelets, the percentage difference was 6.8%.

**Table 2: Mean Values ( $\pm$ SD) of Plasma Electrolytes after 3, 10, and 15 Days for All Groups.**

Groups	calcium			Magnesium			Sodium			Potassium		
	3	10	15	3	10	15	3	10	15	3	10	15
Control	11.2 $\pm$ 0.5			2.4 $\pm$ 0.21			134 $\pm$ 6.7			4.5 $\pm$ 0.23		
Ginkgo biloba	10.8 $\pm$ 0.4	10.6 $\pm$ 0.31	10.5 $\pm$ 0.5	2.4 $\pm$ 0.11	2.4 $\pm$ 0.21	2.3 $\pm$ 0.3	135 $\pm$ 6.3	136 $\pm$ 6.1	133 $\pm$ 3.3	4.6 $\pm$ 0.24	4.7 $\pm$ 0.11	4.4 $\pm$ 0.11
Cisplatin	10 $\pm$ 0.41	9.0 $\pm$ 0.41	8.4 $\pm$ 0.41	2.5 $\pm$ 0.10	2.4 $\pm$ 0.31	2.2 $\pm$ 0.11	136 $\pm$ 3.4	133 $\pm$ 5.3	135 $\pm$ 4.6	4.4 $\pm$ 0.21	4.6 $\pm$ 0.23	4.7 $\pm$ 0.2
Cisplatin + G.b	10 $\pm$ 0.5	10.2 $\pm$ 0.23	9.5 $\pm$ 0.2	2.3 $\pm$ 0.2	2.4 $\pm$ 0.31	2.4 $\pm$ 0.31	133 $\pm$ 6.1	133 $\pm$ 4.6	133 $\pm$ 5.4	4.6 $\pm$ 0.24	4.5 $\pm$ 0.2	4.9 $\pm$ 0.11
Liposomal cisplatin	10.5 $\pm$ 0.2	10.5 $\pm$ 0.34	9.5 $\pm$ 0.3	2.5 $\pm$ 0.22	2.3 $\pm$ 0.21	2.5 $\pm$ 0.4	131 $\pm$ 4.4	130 $\pm$ 6.8	134 $\pm$ 5.1	4.9 $\pm$ 0.13	5 $\pm$ 0.31	4.8 $\pm$ 0.2
Lipo cisplatin + G.b	10.9 $\pm$ 0.3	10.9 $\pm$ 0.2	11 $\pm$ 0.1	2.1 $\pm$ 0.3	2.2 $\pm$ 0.32	2.4 $\pm$ 0.22	130 $\pm$ 5.6	131 $\pm$ 5.3	132 $\pm$ 3.3	4.8 $\pm$ 0.22	4.7 $\pm$ 0.12	5 $\pm$ 0.3

**Table 3: Mean Values ( $\pm$ sd) of Hematological Parameters after 3, 10, and 15 Days for All Groups.**

Group	Wbc ( $10^3$ )			Rbc ( $10^6$ )			Hb			Platelets ( $10^3$ )		
	3	10	15	3	10	15	3	10	15	3	10	15
Control	10 $\pm$ 0.5			4.5 $\pm$ 0.18			13 $\pm$ 0.23			3.65 $\pm$ 1.8		
Ginkgo biloba	9.5 $\pm$ 0.5	10 $\pm$ 0.3	10 $\pm$ 0.3	4 $\pm$ 0.13	4.4 $\pm$ 0.14	4.3 $\pm$ 0.12	12.8 $\pm$ 0.2	13 $\pm$ 0.33	13 $\pm$ 0.32	3.60 $\pm$ 1.4	3.50 $\pm$ 1.3	3.60 $\pm$ 1.1
Cisplatin	7.0 $\pm$ 0.3	6.0 $\pm$ 0.25	5.800 $\pm$ 0.31	3.7 $\pm$ 0.08	3.5 $\pm$ 0.05	3.2 $\pm$ 0.12	10.2 $\pm$ 0.12	10.4 $\pm$ 0.25	10 $\pm$ 0.16	2.25 $\pm$ 4.5	1.90 $\pm$ 5.7	1.80 $\pm$ 4.8
Cisplatin +G.b	6.8 $\pm$ 0.21	6.6 $\pm$ 0.11	6.200 $\pm$ 0.23	3.8 $\pm$ 0.05	3.8 $\pm$ 0.14	3.7 $\pm$ 0.14	10.5 $\pm$ 0.05	11.1 $\pm$ 0.11	10.9 $\pm$ 0.12	2.50 $\pm$ 5.1	2.00 $\pm$ 3.4	2.25 $\pm$ 4.3
Liposomal cisplatin	8.6 $\pm$ 0.21	8.6 $\pm$ 0.11	8.8 $\pm$ 0.21	3.8 $\pm$ 0.12	3.7 $\pm$ 0.11	3.9 $\pm$ 0.13	10.7 $\pm$ 0.084	11.3 $\pm$ 0.10	11.4 $\pm$ 0.09	2.65 $\pm$ 3.4	2.90 $\pm$ 2.1	2.90 $\pm$ 1.6
Lipo cisplatin + G.b	9.8 $\pm$ 0.11	9.1 $\pm$ 0.3	9.500 $\pm$ 0.32	3.9 $\pm$ 0.11	3.9 $\pm$ 0.09	4.1 $\pm$ 0.08	11 $\pm$ 0.13	11.5 $\pm$ 0.31	11.9 $\pm$ 0.35	3.35 $\pm$ 2.6	3.35 $\pm$ 1.8	3.40 $\pm$ 1.6

### Discussion

In this study, we report the properties of a liposomal formulation of cisplatin developed in order to reduce the systemic toxicity of cisplatin. Liposomal cisplatin was shown to be less toxic than cisplatin alone (*Teni Boulikas, 2007*). The lower toxicity of Liposomal cisplatin compared to cisplatin may be due to alterations in its pharmacokinetics, preferential localization to tumors containing compromised vasculature and differences in cellular uptake. Based on the calorimetric studies, we could propose that cisplatin and Ginkgo biloba can be easily incorporated into the DSPC bilayers, thus affecting its thermotropic phase behavior.

The main transition temperature of DSPC liposomes (79°C) is strongly shifted to lower temperature (60°C) for DSPC liposomes encapsulating cisplatin in the presence of Ginkgo biloba, which suggests that Ginkgo biloba has a significant effect on the acyl chains of DSPC bilayers and its presence decreases the transition cooperativity of lipid acyl chains. The change in phase transition temperature suggests that the incorporated Ginkgo biloba could be resident near the interface region and within the hydrophobic core thus giving rise to Ginkgo biloba enriched microdomains. The above observation is concordant with the finding of

*Ladbrooke, 1968* that the interaction of the encapsulated drug with the lipid components of liposomes may alter the physicochemical properties of liposomes, which in turn would influence the drug transfer from the liposomes.

Our study showed that all ERG parameters and many blood constituents were affected by the administration of cisplatin which suggests functional changes in both of them. The hematological parameters showed that there is a decrease in the level of Hb, RBCs, and WBCs, platelets and calcium. No changes were observed in the levels of sodium, potassium and Magnesium that are in accordance with the finding of *Özlem, 2008*. This observation could be attributed to the low dose used in this study (1mg/kg), while the therapeutic dose is 5mg/kg. This reduction of hematological parameters (RBCs, WBCs, Hb, platelets and calcium) manifested by the administration of cisplatin may indicate the emergence of complications like myelosuppression anemia, thrombocytopenia, leukopenia and hypocalcaemia which are usually associated with cancer chemotherapy (*Prasad, 2006*). The obtained results revealed the hematotoxicological effects of cisplatin which may be due to the formation of reactive oxygen species (ROS) (*Shafaq and Tabassum, 2010*) which consequently altered the



membrane permeability, thus disturbing the cations homeostasis. ROS has high affinity towards the thiol group, so, it oxidizes the thiol group(-SH) of Na<sup>+</sup>-K<sup>+</sup> ATPase and decreases its activity (*Shafaq and Tabassum, 2011*). Also the effect may be due to the depletion of glutathione (GSH) which is an important cellular antioxidant and is known to have protective functions in the cells against toxic effects of drugs and metals (*Prasadet, 2006*).

Calcium plays a key role in regulating cell metabolic processes by stimulating or inhibiting key enzymes (*Zhang and Lindup, 1996*). It has been reported that hypocalcaemia is a known side effect in cisplatin chemotherapy (*Arany and Safirstein, 2003*). Hypocalcaemia is caused by the excessive urinary loss of calcium and its decreased renal uptake during treatment with high doses of cisplatin. Proximal tubular damage which is reported to happen in cisplatin therapy leads to decreased reabsorption of cations; as a result, it is expected to decrease calcium levels.

Beside the hematological toxicity there are also some undesirable effects on the electroretinogram of rabbits after the administration of cisplatin. These effects are in the form of reduced amplitude and increased implicit time of the ERG waves, which suggests functional changes in the retina. This finding is in agreement with *Omoti et al., (2006); Gonzalez (2001); Katz (2003) and Hilliara (1997)*. The ERG a-wave is generated by photoreceptors in the outer retinal layer, which receives blood from the choroidal vessels, while the b-wave is generated in the inner layer that is supplied mainly from the retinal circulation, thus the b-wave is useful for the evaluation of retinal circulatory disturbance. The retina is a vascularized neural structure that is composed of blood vessels, neurons, glia and microglia. ERG is performed by several different types of neurons within the retina. Photoreceptors capture photons and transduce light energy to chemical signals. The photoreceptors form synapses with bipolar and horizontal cells, (*Masland, 2001; Newman and Zahs, 1998*). The function of the neurons and the blood vessels is integrated by the glial cells (astrocytes and Müller cells) which extend small projections that envelope the blood vessels in the inner layer and convey nutrients from the blood vessels to the neurons. The Müller cells support nearly the entire thickness of the retina and regulate the local ionic environment and the electrical properties of the neurons (*Thomas, 2005*). Blood plays an important role in the retinal function and the changes in the ERG after administration of cisplatin can be attributed to the changes in blood. By correlating the present results of both blood and ERG, it is concluded that the changes in the ERG may be due to the anemia which is a drop in the total number of RBCs and a decrease of hemoglobin that carries oxygen to the retina leading to retinal ischemia and

consequently, the b-wave is reduced (*block, 1998; Kyoung 1988; Matsumdo 2011*). Also, the resultant decrease in platelets (thrombopenia) is one of the causes of retinopathy which leads to retinal /vitreal hemorrhage or retinal detachment, that is in both cases there will be a decrease in the a- & b-wave amplitudes and prolongation in their implicit times (*Helga et al., 2011; Regillo et al., 1999*). There is a proved link between inflammatory diseases and intracellular oxidative stress which results from the ROS generated by cisplatin that may attack DNA, protein or lipid cellular components which may explain the change in ERG (*Marikoet et al. 2009; Valko et al. 2007*). The administration of oral Ginkgo biloba during cisplatin treatment showed an improvement in the undesirable effects, this may be a result of the anti oxidative activity of ginkgo biloba that restored the membrane permeability which was altered by the free radicals (*Shafaq and Tabassum, 2010*), and /or reduction of platelet – activating factor and improvement of peripheral circulation (*Gamal et al., 2011; Chung et al., 1999*). By applying liposomal cisplatin, a significant improvement was observed in all parameters of both blood & ERG. Encapsulation of anticancer drugs into nanoparticle formulations (liposomal form) can alter the bio distribution, lower the side effects, minimize the toxic exposure to normal tissues while maximizing tumor uptake and penetration of the drug (*Boulikas, 2004, 2007*). Because of the lipid shell, liposomal cisplatin does not harm the cells of the kidney and other normal tissues to cause nephrotoxicity (*Devarajan et al, 2004*) and other side effects, also that may explain the increase of total calcium in blood as compared to group III which was administered cisplatin only.

Our targets, which were lessening the side effects of cisplatin, were reached in the last group; the combination of liposomal cisplatin and Ginkgo biloba gave the closest result to normalcy as possible.

### Conclusion

The current data shed new light that proposes a new treatment regimen in which cisplatin is replaced by liposomal cisplatin combined with Ginkgo biloba to reduce undesirable side effects and to produce similar efficiency to that of cisplatin. This preclinical study needs more researches to evaluate this new technique.

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