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ANTITUMOR ACTIVITY OF RUTIN-CISPLATIN IN COMBINATION AND ITS PROTECTIVE EFFECT AGAINST HEMATOTOXICITY

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ABSTRACT: Rutin, a naturally occurring flavonoid, possesses many pharmacological properties. Cisplatin is a well-known cancer chemotherapeutic drug. However, its full therapeutic efficacy is hindered due to development of various adverse side-effects in the host. The present study was focused on to evaluate rutin-cisplatin combination therapeutic efficacy and its protective role against cisplatin-induced hematotoxicity in ascites Dalton's lymphoma (DL) bearing mice. Ascites Dalton's lymphoma tumor-transplanted mice were treated with rutin or cisplatin alone and in the combination. The percentage increase in life span (ILS) of the hosts, cell viability, fluorescence-based apoptosis, glutathione and hematological parameters were determined under different treatment conditions. It was observed that cisplatin in combination with rutin has better therapeutic potential against murine ascites Dalton's lymphoma showing about 57 percent more ILS than cisplatin alone. The increased ILS observed during combination treatment could involve increased cytotoxicity and apoptosis in DL cells as compared to alone treatment. The combination treatment caused further decrease in reduced glutathione (GSH) level in tumor cells which could weaken its defensive ability and also assist in tumor cells death thereby increasing the host's survivability. Cisplatin treatment of the tumor-bearing mice caused a decrease in erythrocytes and leukocytes count and hemoglobin content which could lead to develop hematotoxicity in the hosts. However, the combination treatment showed a noteworthy improvement in all these hematological parameters suggesting a protective ability of rutin against cisplatin-induced hematotoxicity.

KEYWORDS: Cisplatin, rutin, antitumor activity, hematotoxicity

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1. INTRODUCTION

Cancer and its treatment is one of the major health-care issues for human and demands an optimistic approach for its remedy. As per the facts and figures of American Cancer Society (2018), the global cancer burden indicates that cancer accounts for about 1 in every 7 deaths worldwide – more than HIV/AIDS, tuberculosis, and malaria combined and by 2030, the global burden is expected to reach 21.6 million new cancer cases [1]. The main conventional methods for the cancer treatment are surgery, radiotherapy and chemotherapy which could be used singly or in combination. Out of these methods, chemotherapy is an effective treatment mode which includes hundreds of drugs against wide variety of cancers [2-4]. Many compounds with metal ions have been used for various medicinal purposes including cancer [5]. *Cis*-dichlorodiammineplatinum(II), (CDDP, cisplatin) is a water-soluble, square planar co-ordination complex containing a central platinum atom surrounded by two chloride atoms and two ammonia moieties in *cis*-configuration (Figure 1A). Cisplatin is one of the most widely used anticancer drugs for testicular, ovarian, bladder, cervical, head and neck cancers [6]. The anticancer activity of cisplatin has been credited to its DNA binding ability and the formation of covalent cross-links that subsequently result in inhibition of DNA replication and transcription [7]. However, full therapeutic efficacy of cisplatin is hampered due to the development of various side effects in the host and/or the acquired drug resistance by the cancer cells [8, 9]. The prominent side effects developed after cisplatin treatment are nephrotoxicity, myelotoxicity, haemototoxicity, neurotoxicity, ototoxicity etc. [10]. In an attempt to overcome diverse toxicities/side effects caused by cisplatin in the hosts many agents such as phenoxodiol, genistein and gingerol, baicalein, ascorbic acid, naringenin-oxime etc. have been used to lessen toxicities without compromising on its therapeutic efficacy in the hosts [11-15]. Flavonoids, poly-phenolic compounds, are one of the important classes of plant derived chemicals having wide-ranging biological properties including anti-bacterial, antiviral, anti-cancer, anti-inflammatory, hepato-protective and antioxidant effects [16]. Rutin, also called as rutoside, quercetin-3-rutinoside and sophorin, is a citrus flavonoid glycoside found in passion flower, buckwheat, tea, and apple [17]. The name ‘rutin’ comes from the plant *Ruta graveolens*, which also contains rutin. Chemically it is a glycoside comprising of flavonolic aglycone quercetin along with disaccharide rutinose (rhamnose and glucose) (Figure 1B). Various studies have proved that flavonoids have ability to protect the genome from chemical carcinogens and are also able to prevent and cure the cancers. Rutin has demonstrated various pharmacological properties, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities [18-21]. Many studies have also reported the protective effects of rutin against various drug induced toxicities in the hosts [22-24]. Other studies have reported that rutin alters signal transduction [25], causes activation of transcription factors and gene expression [26] and may also protect DNA by interacting with carcinogens that have escaped detoxification processes [27, 28]. Recently, the ameliorative

effects of rutin against isoniazid-induced alterations in certain hematological and biochemical parameters has been reported [29]. Keeping in view the various aspects of beneficial pharmacological properties of rutin, present study was undertaken to evaluate the therapeutic efficacy of rutin alone and in combination with cisplatin against murine ascites Dalton's lymphoma and also to assess its protective role against cisplatin-induced hematotoxicity in the same tumor-bearing hosts.

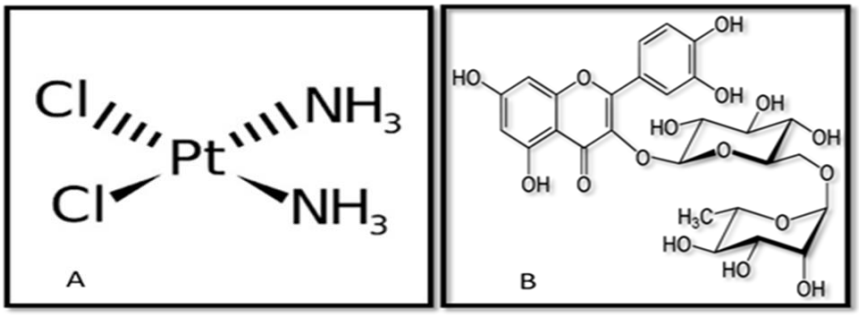

Figure 1: Chemical structure of cisplatin (A) and rutin (B)

2. MATERIALS AND METHODS

2.1 Chemicals

Rutin (CAS No. 153-18-4, rutin hydrate) with ≥94% purity was purchased from Sigma (St. Louis, MO, USA). Cisplatin solution (1 mg/ml of 0.9%, NaCl) was obtained from Biochem Pharmaceutical Industries, Mumbai, India. RBC and WBC diluting fluids and all other chemicals used in the experiments were of analytical grade and purchased from SRL Pvt. Ltd., Mumbai, India.

2.2 Animals and tumor maintenance

Inbred Swiss albino mice colony is being maintained under standard laboratory conditions, keeping 4-5 mice per cage with free access to standard food pellet diet and drinking water *ad libitum*. Ascites Dalton's lymphoma (DL) malignant tumor is being maintained *in vivo* by serial intra-peritoneal (i.p.) transplantation of viable tumor cells (1×10^7 cells in 0.25 mL phosphate-buffered saline (PBS), pH 7.4) in 10-12 weeks old Swiss albino mice of both sexes weighing 25-30g (Figure 2). Tumor-transplanted hosts usually survive for 19-21 days. The maintenance and use of the mice and the experimental protocol of the present study has been approved by the Institutional ethical committee (IEC) of North-Eastern Hill University, Shillong, India.

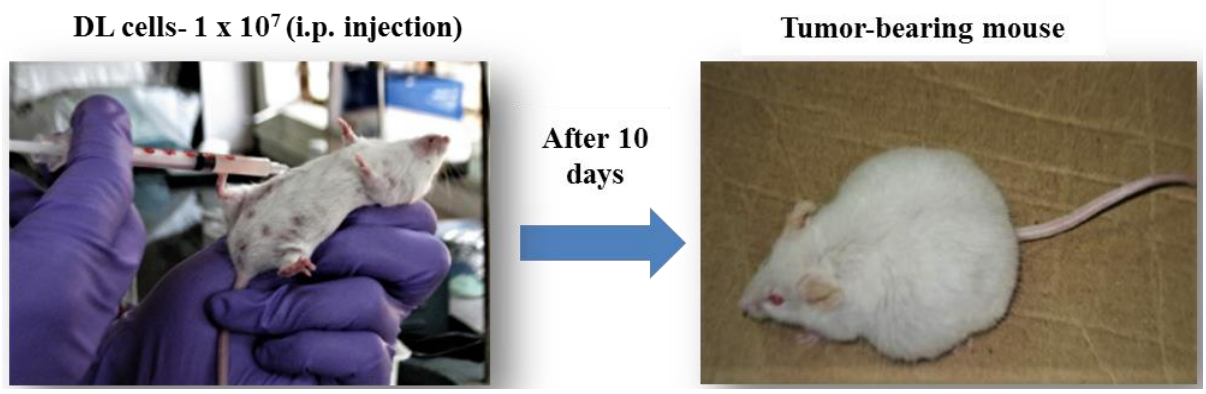

Figure 2: Procedure of tumor transplantation and its maintenance (i.p.=intra-peritoneal)

2.3 Drug treatment schedule and antitumor activity study

Rutin solution was prepared a fresh in dimethyl sulfoxide (DMSO) (1 mg/20 mL), then diluted in PBS to get the desired concentration, where the final concentration of DMSO did not exceed 0.5%. Based on the standardization of the doses in the preliminary experiments and other report [30], the therapeutic dose of rutin was selected as 30mg/kg body weight for the treatment. Similarly, the dose of cisplatin (8mg/kg body weight) was selected as per earlier study in the lab [31]. Tumor-transplanted mice were randomly divided into four groups (10 mice/group) as follows:

Group I: mice served as tumor-bearing control received drug vehicle only.

Group II: mice were treated with rutin (30mg/kg body weight, i.p.) on 8^{th} and 10^{th} day post-tumor transplantation

Group III: mice were treated with single therapeutic dose of cisplatin (8mg/kg body weight, i.p.) on 10^{th} day post-tumor transplantation.

Group IV: mice were treated with rutin on 8^{th} and 10^{th} day followed by a single dose of cisplatin on the 10^{th} day post-tumor transplantation keeping a gap of 6 h post rutin treatment.

The experiments were performed in triplicates, in three independent sets. Figure 3 summarizes the drugs treatment schedule.

The deaths, if any, of the hosts were recorded daily and the survival patterns of the hosts in different groups were determined. The antitumor efficacy was determined as percentage increase in life span (%ILS) of the hosts using following formula [32]:

$$\text{Increase of life span (ILS)} = \left[\frac{\text{MST of treated group} - \text{MST of control group}}{\text{MST of control group}}\right] \times 100$$

$$\text{where MST} = \frac{\sum \text{survival time (days) of each mice in a group}}{\text{Total number of mice}}.$$

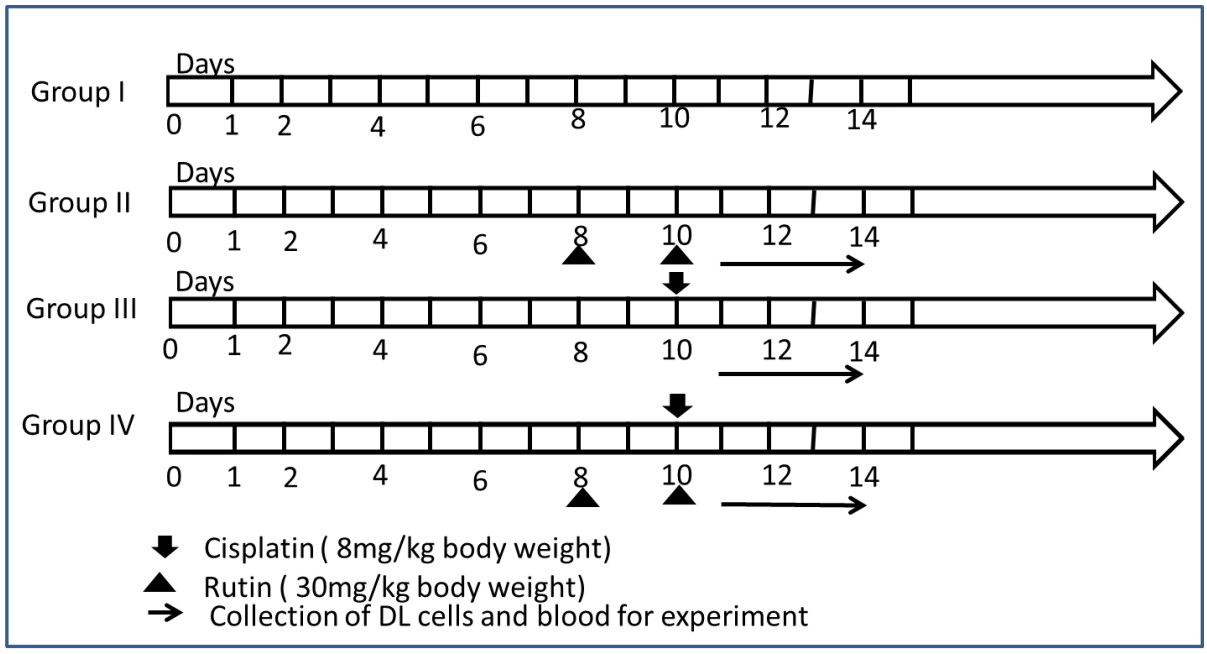

Figure 3: Summary of drugs treatment schedule

Drugs are termed active, if the obtained %ILS value is ≥ 20%. Further, in other set of experiment, after 24, 48, 72 and 96h of the last treatment (i.e. on 11, 12, 13 and 14th day post tumor transplantation), two animals from each group were sacrificed by cervical dislocation and ascites DL cells and blood samples were collected for other experimental analysis.

2.4 Trypan blue exclusion test

Cell viability of DL cells was checked using trypan blue exclusion test [33]. Briefly, after 24, 48, 72 and 96 h treatment of DL bearing mice in different groups, DL cells were collected and washed twice with PBS. Then, aliquot of the cell suspension was mixed with an equal volume of trypan blue (0.4% in PBS) and kept for 2 minutes. Viable (unstained cells) and non-viable (stained cells) were counted with a Neubauer haemocytometer under light microscope (Meiji). The percentage of cells viability was calculated by monitoring 10-15 different view-fields using the following formula:

$$\%\text{Viability} = \frac{\text{Total No. of viable cells}}{\text{Total No. of viable and non-viable cells}} \times 100$$

2.5 Fluorescence based apoptosis study

Apoptosis was determined in DL cells collected from mice under different treatment conditions using acridine orange and ethidium bromide (AO/EB) staining method as described by [34, 35]. After treatment, the DL cells were collected from mice at different time intervals. Then, tumor cells were washed twice with PBS and treated with AO/EB (100µg/ml PBS of each dye) for 5 minutes. The cells in different treatment groups were thoroughly examined under fluorescent microscope (Leitz) and photographed using digital camera (A1000IS-Canon). One thousand cells were analyzed

and percentage of apoptotic cells was calculated from 15 selected view fields under microscope and compared with that of control.

2.6 Reduced glutathione (GSH)

Total reduced glutathione (GSH) in DL cells was determined using the method of [36]. Briefly, 5% homogenates of DL cells were prepared in 0.02 M EDTA (pH 4.7). Total GSH was determined by adding the cells homogenate or pure reduced form of glutathione (100 µl) to 0.9 ml of 0.02 mol/L EDTA, pH 4.7 and 1ml of 0.2 mol/L Tris–EDTA buffer, pH 8.2, and followed by 20µl of Ellman's reagent (10 mmol/L DTNB in methanol). After 30 minutes of incubation at room temperature, the reaction mixture was centrifuged at 3000xg for 15 minutes and the absorbance of the clear supernatant was read against a reagent blank at 412 nm in a Varian Carey-50 spectrophotometer.

2.7 Hematological studies

Blood samples from the mice in different treatment groups were collected by puncturing the retro-orbital venous sinus using sterilized glass capillaries into a blood collection tube containing K2 EDTA as an anticoagulant. The hematological parameters i.e., haemoglobin (Hb) content, red blood cells (RBCs) counts, white blood cells (WBCs) counts and packed cell volume (PCV) were determined using the method of [37].

2.8 Statistical analysis

All the values obtained from the study have been expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to evaluate the difference among multiple groups followed by a post hoc test (Tukey test).The analysis was carried out using Origin 8.0 software.

P-value ≤ 0.05 was considered as statistically significant for all comparisons.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Antitumor activity study

Control tumor-transplanted mice (Group I) survived for about 19-21 days. The mean survival time of mice treated with rutin (RUT) alone (Group II) or *cis*-diamminedichloroplatinum (II) **(**CDDP) alone (Group III), was increased to about 31 days and 43 days respectively. However, the hosts' survivability was further increased to about 54 days in the combination treated group of mice (Group IV). The % ILS obtained in the RUT, CDDP alone and RUT plus CDDP treated group of mice was about 60 %, 127 % and 184 % respectively (Table 1). Thus, CDDP in combination with RUT showed about 57 percent more ILS than that for CDDP alone treatment.

Table 1: Survival patterns of DL-bearing mice under different treatment conditions. Results are expressed as mean ± S.D. The significance of changes between control and different treated groups was tested by one-way ANOVA, n= 3, *P≤ 0.05 as compared to the corresponding control; #P≤ 0.05 as compared to CDDP. Control= Untreated tumor-bearing mice; RUT= Rutin; CDDP= *cis*-diamminedichloroplatinum (II); ILS= increase in life span and i.p.=intraperitoneal.

Treatment groups	Day of Treatment	Route of Treatment	Survival Days (MST ± S.D.)	ILS (%)
Group I(Control)	_	_	19 ± 1.9	_
Group II	8th and 10thday (RUT)	i.p.	30.5 ± 1.29*	60.53
Group III	10th day (CDDP)	i.p.	43.2 ± 3.4*	127.37
Group IV	8th and 10thday (RUT) plus 10th day (CDDP)	i.p.	54 ± 2.2*#	184.21

3.1.2 Trypan blue exclusion test

Trypan blue exclusion test showed significant decrease in viability of DL cells in each treatment groups in a time-dependent manner (Figure 4). However, in combination treatment group loss in cell viability (dead cells) was maximum (~78%) which was more compared to the alone treatment (RUT and CDDP) group (~60% and 65% respectively) (Figure 5).

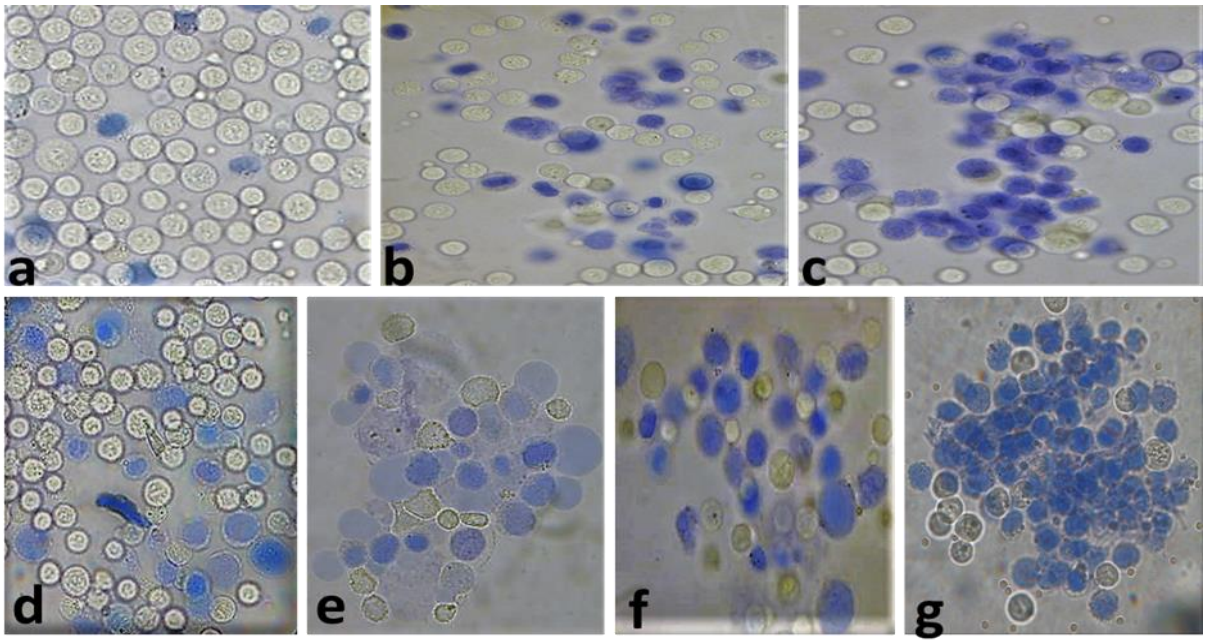

Figure 4: Representative photographs of viable and dead DL cells obtained by trypan blue exclusion test. (a) Control DL cells treated with drug vehicle only; (b) RUT alone treated DL cells after 96h; (c) CDDP alone treated DL cells after 96h; (d-g) combination treated (RUT + CDDP) DL cells for 24-96h duration. Viable DL cells are colourless whereas dead cells stained blue. RUT=Rutin; CDDP=*cis*-diamminedichloroplatinum (II).

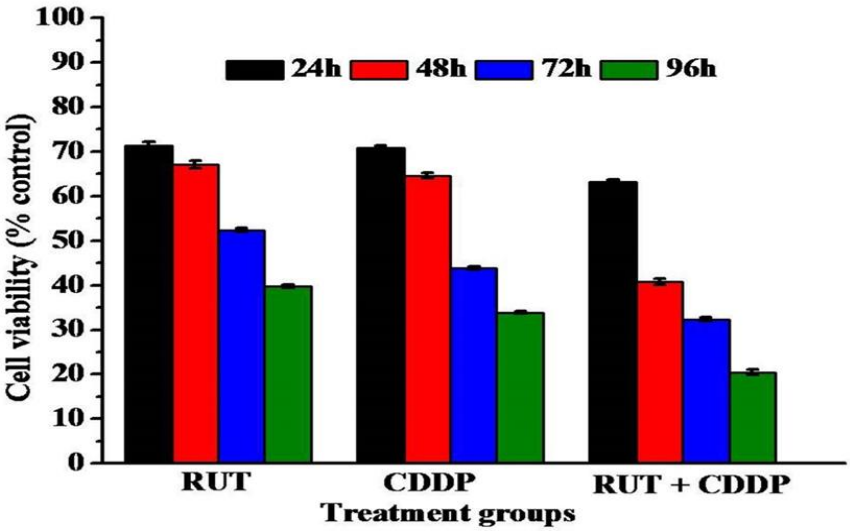

Figure 5: Change in the viability of DL cells under different treatment conditions. Results are expressed as mean ± S.D. n=3; RUT=Rutin; CDDP=*cis*-diamminedichloroplatinum (II).

3.1.3 Fluorescence based apoptosis study

The viable DL cells' nuclei stain green, due to permeability of only acridine orange whereas apoptotic cells appear red/orange due to co-staining of acridine orange and ethidium bromide dyes. The prominent apoptotic features such as membrane blebbing (MB), apoptotic bodies (AB), fragmented nuclei (FN) and chromatin condensation (CC) were observed in all the treated groups but the maximum changes were observed in the combination treatment (Figure 6). The maximum apoptotic cells were obtained in combination treatment (~68%) as compared to the respective alone treatment (~53% and ~64% respectively) (Figure 7).

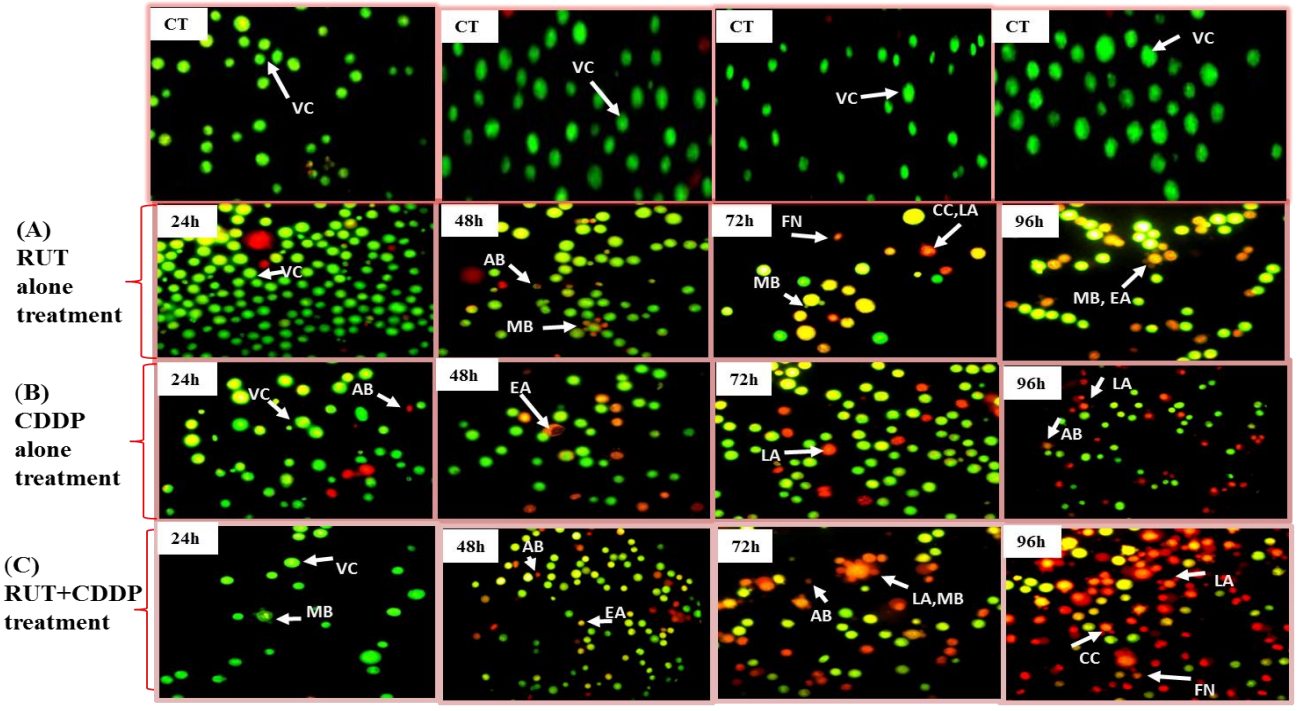

Figure 6: Morphological features of apoptotic and viable Dalton's lymphoma (DL) cells stained

with acridine orange and ethidium bromide. (A) DL cells from tumor-bearing mice treated with RUT alone showing 24-96h duration (B) DL cells from tumor-bearing mice treated with CDDP alone for 24-96h. (C) DL cells from tumor-bearing mice treated with combination of RUT plus CDDP for 24-96h. Prominent apoptotic features such as membrane blebbing (MB), apoptotic bodies (AB), fragmented nuclei (FN), chromatin condensation (CC), early apoptosis (EA) and late apoptosis (LA) were noted. Green nuclei are viable cells (VC) and red/orange nuclei indicate apoptotic cells (EA/LA). CT=Control i.e., DL cells from tumor-bearing mice without any treatment; RUT=Rutin; CDDP=*cis*-diamminedichloroplatinum (II). Scale bar of each picture is 50µm (magnification: x40).

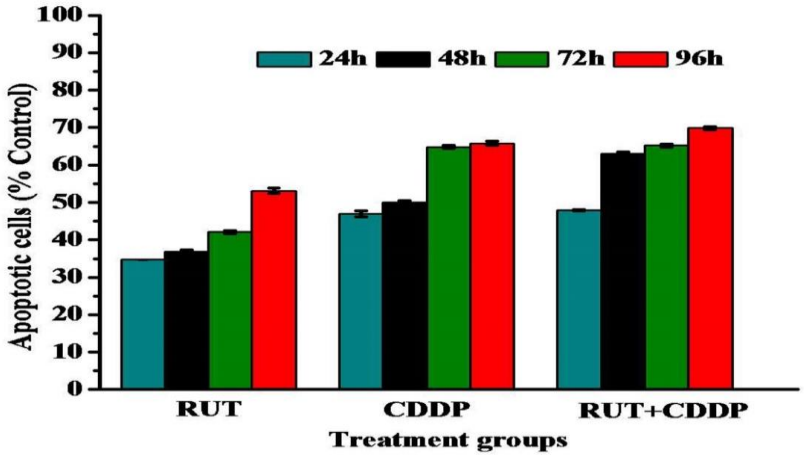

Figure 7: The apoptotic DL cells (% control) obtained in different treated groups. Results are expressed as mean ± S.D. n=3; RUT=Rutin; CDDP=*cis*-diamminedichloroplatinum (II).

3.1.4 Reduced glutathione (GSH)

As compared to corresponding control, RUT, CDDP alone as well as combination treatment caused a decrease in GSH level in DL cells. However, in combination treatment, the decreased GSH level was much more than that for alone treatment (Table 2).

Table 2: Changes in GSH content (µmol/g wet wt) in DL cells from tumor-bearing mice under different treatment conditions. Control=Untreated tumor-bearing mice. RUT=Rutin; CDDP=*cis*-diamminedichloroplatinum (II). Results are expressed as mean± S.D. ANOVA, n=3, *P≤0.05 as compared to the corresponding control; #P≤ 0.05 as compared to CDDP.

Treatment duration	Control	RUT alone treatment	CDDP alone treatment	RUT+CDDP treatment
24 h	4.11 ± 0.59	2.46 ± 0.19*	2.85 ± 0.02*	2.78 ± 0.50*
48 h	3.11 ± 0.40	2.89 ± 0.23	2.92 ± 0.05	2.68 ± 0.40#*
72 h	3.75 ± 0.23	2.74 ± 0.08*	2.94 ± 0.07*	1.91 ± 0.36#*
96 h	3.09 ± 0.42	2.52 ± 0.13*	2.77 ± 0.10*	1.74 ± 0.53#*

3.1.5 Hematological studies

As compared to the Hb content, RBC count and WBC count in normal mice, there was a decrease in their values in control tumor-bearing hosts. As compared to corresponding control, RUT alone treatment caused an increase in the Hb content at 72-96h (~30%) (Table 3). CDDP alone treatment caused an overall decrease in the Hb content while combination treatment caused an increase with betterment in Hb level thereby regaining Hb concentration. As compared to control and CDDP alone, RBCs counts were noted to be more in RUT alone and in combination treatment. As compared to corresponding control, WBCs count decreased in RUT as well as CDDP alone treatment. As compared to CDDP alone treatment, combination treatment caused a significant increase in the number of WBCs in a time-dependent manner. As compared to corresponding control, significant decrease in the PCV was noted at 24-96 h of RUT or CDDP alone treatment. However, as compared to CDDP alone, combination treatment significantly increased the PCV values during 24-96 h of treatment (Table 3).

Table 3: Hematological parameters in the normal and tumor-bearing mice under different treatment conditions. Hb=Haemoglobin, RBC=Red blood cells, WBC=White blood cells, PCV=Packed cell volume, Normal=Mice without any treatment or tumor-bearing. Control=Untreated tumor-bearing mice, RUT=Rutin; CDDP=*cis*-diamminedichloroplatinum (II). Results are expressed as mean ± S.D. ANOVA, n=3, *P≤0.05 as compared to corresponding control; #P≤ 0.05 as compared to CDDP.

Treatment groups	Hb (g/dl)	RBC (x10^{12}/l)	WBC (x10^{9}/l)	PCV (%)
Normal	14.56 ± 0.23	7.26 ± 0.62	5.48 ± 2.60	42.66 ± 1.93
Control: 24 h	11.22 ± 1.05	4.60 ± 0.04	8.62 ± 0.82	36.52 ± 1.18
48 h	10.66 ± 0.49	3.96 ± 0.60	9.05 ± 0.39	35.20 ± 0.13
72 h	9.75 ± 0.42	4.96 ± 0.40	9.86 ± 0.42	35.90 ± 0.56
96 h	9.04 ± 1.13	4.72 ± 0.16	10.23 ± 0.79	33.73 ± 1.60
RUT alone: 24 h	9.60 ± 1.49	3.16 ± 2.44	7.88 ± 1.01	30.09 ± 0.95*
48 h	10.30 ± 0.79	5.34 ± 0.26*	8.23 ± 0.66	30.23 ± 0.82*
72 h	12.92 ± 1.82*	7.08 ± 1.47*	8.96 ± 0.06	31.48 ± 0.43*
96 h	11.56 ± 0.46*	6.84 ± 1.23*	10.50 ± 1.60*	32.39 ± 1.34
CDDP alone: 24 h	8.50 ± 0.24*	5.14 ± 0.95	8.42 ± 0.61*	29.28 ± 0.22*
48 h	8.55 ± 0.29*	5.08 ± 0.89	8.56 ± 0.47*	29.87 ± 0.36*
72 h	8.43 ± 0.17*	3.38 ± 0.81*	9.20 ± 0.16*	30.50 ± 0.99*
96 h	7.58 ± 0.68*	3.16 ± 1.03*	9.96 ± 0.92*	28.36 ± 1.14*
RUT+CDDP :24 h	10.37 ± 2.03#	5.34 ± 1.23*	10.56 ± 0.57#*	34.43 ± 2.15#*
48 h	11.96 ± 0.44#*	7.08 ± 0.50#*	10.23 ± 0.90#*	35.21 ± 1.37#*
72 h	13.40 ± 0.99#*	6.64 ± 0.06#*	11.16 ± 0.03#*	37.22 ± 0.63#*
96 h	13.89 ± 1.48#*	7.24 ± 0.67#*	12.57 ± 1.44#*	39.48 ± 2.89#*

3.2 Discussion

Cis-dichlorodiammineplatinum (II), (cisplatin, CDDP) has been frequently used in combination with one or more drugs in cancer chemotherapy with results showing better therapeutic efficacy and decreased toxicities [38, 39]. Rutin, a poly-phenolic bioflavonoid has shown wide range of pharmacological applications due to its significant antioxidant properties and its use is advantageous over other flavonoids as it is a non-toxic and non-oxidizable molecule [40]. To establish the efficacy of any anticancer drug, determination of the prolongation of life span of the treated tumor-bearing hosts as compared to control has been used as an important criterion [41, 42]. The results of the survival patterns of the hosts in different groups showed the significant increase in survivability of the hosts after combination treatment as compared to the mice treated with either compound alone (Table 1) which may suggest that combination treatment with rutin plus cisplatin could be a better therapeutic approach against murine ascites Dalton's lymphoma. Moreover, the viability of DL cells decreased more prominently in combination treatment as compared to that of alone treatment (Figure 5) which may suggest that this combination is more cytotoxic to DL cells which could effectively facilitate to increase the survivability of tumor-bearing mice. Apoptosis is a type of genetically regulated programmed cell death that controls the development of multicellular organisms and tissues by eliminating physiologically redundant, physically damaged and abnormal cells [43]. Apoptosis is the major cause of cell death induced by antitumor drugs and radio-sensitization drugs and the efficacy of anticancer drugs is measured by their ability to selectively promote apoptosis in cancer cells [44]. The fluorescence-based study for apoptosis using AO/EB staining showed that combination treatment significantly increased chromatin condensation, nuclei fragmentation, appearance of more apoptotic bodies and membrane blebbing in DL cells (Figure 6). The number of apoptotic cells was also much more in combination treatment as compared to the alone treatment which further corroborates the viability results that the combination treatment has high cytotoxicity potential against DL cells (Figure 7). Glutathione (GSH), a tri-peptide, plays important roles in antioxidant defense, nutrient metabolism, and regulation of cellular processes, including cell differentiation, proliferation and apoptosis [45]. The lower intracellular GSH levels decrease cellular antioxidant capacity whereas elevated GSH levels generally increase antioxidant capacity and resistance to oxidative stress [46]. The changes in the level of GSH in the DL cells showed significant decrease in combination treatment as compared to other groups which could weaken its defensive ability and assist in the tumor cells death thereby increasing the host's survivability (Table 2). Cisplatin alone treatment caused significant reduction in RBC counts with subsequent decline in Hb concentration and PCV which is a known side-effects induced by cisplatin in the hosts [47]. Therapeutic doses of cisplatin are evidently toxic to bone marrow cells and probably can trigger apoptosis and affect cell cycle causing an anemia and a decrease in WBC count [48]. In present study, treatment with the combination of rutin and cisplatin showed significant

improvement in all the hematological values indicating its potential to reduce cisplatin-induced hematotoxicities (Table 3). In a study Longchar and Prasad [47] reported that combination treatment with ascorbic acid plus cisplatin has better therapeutic efficacy against murine ascites DL in comparison to cisplatin alone treatment and simultaneously helps in the recovery in cisplatin-induced hematological values and significant reduction in mutagenic and genotoxic effects [14]. It has been reported that rutin effectively ameliorates the alterations in the hematological parameters through its antioxidant nature [49] and the side-effects of synthetic drugs may be decreased due to flavonoid content in the combinational drugs [50]. Thus, it may be suggested that rutin plus cisplatin combination treatment while illustrating protective ability against cisplatin induced hematotoxicity has promising therapeutic efficacy against cancers in general and murine ascites Dalton's lymphoma in particular.

4. CONCLUSION

Cisplatin in combination with rutin has better therapeutic potential against murine ascites Dalton's lymphoma which could involve increased cytotoxicity and apoptosis in DL cells as compared to alone treatment. The combination treatment caused more decrease in reduced glutathione (GSH) level in tumor cells which could weaken its defensive ability and assist in the tumor cells death thereby increasing the host's survivability. Cisplatin treatment of the tumor-bearing mice developed hematotoxicity in the hosts. However, the combination treatment showed a noteworthy improvement in all the hematological parameters suggesting a protective ability of rutin against cisplatin-induced hematotoxicities. Further, details on the molecular mechanisms behind the therapeutic efficacy and its protective ability should be explored.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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