EXTRACT OF OLIVE (*OLEA EUROPAEA L.*) CURES HEMATOLOGIC TOXICOSIS INDUCED BY DOXORUBICIN

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Abstract

The protective effects of extract of *Olea europaea L* was evaluated in hematological toxicosis induced by Doxorubicin (DOX). Standard Soxhlet apparatus was used to prepare ethanolic extract of *Olea europaea L* leaves. Eighteen Wister rats (male) were incorporated in the study and divided in 3 groups: T* (control group: 2 ml 0.9 % Normal Saline), TP0 (DOX group: 15 mg/kg) and TP1 (extract of *Olea europaea L* + DOX: 200 + 15 mg/kg). The drugs were administered by intra peritoneal route. Blood was withdrawn on 57th day by cardiac puncture and preserved for analysis. Hematological profile included CBC and Platelet count. Hematological analysis revealed that the animals which received Doxorubicin had low levels of Hemoglobin, RBCs, Leukocytes, Neutrophils, Eosinophils, Monocytes, Lymphocytes and Platelets when compared to control group which received normal saline. The levels of Hb and RBCs were low but not manifested in anemia. Neutrophil and Platelet count were drastically low, which shows positive sign of Neutropenia and Thrombocytopenia. The treated group animals which receive extract + DOX showed positive elevation in the levels of CBC and Platelet count. On the basis of hematological report it is concluded that combination of extract of *Olea europaea L* can prevent the toxicosis effects of Doxorubicin.

Introduction

In 1950's Anthracycline (first agent DAUNORUBICIN) from Streptomyces peucetius (soil bacterium) was derived (Di Marco *et al*., 1981). Later, derivative of Daunorubicin was identified i.e. Doxorubicin (DOX), which was more effective anti tumor agent (Arcamone *et al*., 1969). DOX was a potent and highly effective drug in treating many varieties of cancer. Due to its toxic effects on heart, its usage was, however, controlled (Pfeffer *et al*., 2009). The potential mechanisms of cardiac toxicity were free radical production, lipid peroxidation (Pfeffer *et al*., 2009), destruction of mitochondrial assembly (Tokarska-Schlattner *et al*., 2007), release of vasoactive amine (Kotamraju *et al*., 2004) and cellular toxicity. It also reacts with iron to initiate the production of ROS which causes cell death by apoptosis (Kotamraju *et al*., 2004).

Olive (*Olea europaea L*) belongs to Oleaceae family had a long history for its medicinal, nutritional and health benefits. Phenolic constituents including oleuropein, hydroxytyrosol and tyrosol were responsible for most of the olive pharmacologic activities (Ghanbari *et* al., 2012). The extract in a time and dose dependent manner have decreased proliferation and possesses cytotoxic effects (Allouche *et al*., 2011), also inhibits production of Reactive oxygen species. It decreases the production of Thromboxane A2, anti-inflammatory and inhibition of platelet aggregation (Pieroni *et al*., 1996), reduces infarct size (Andreadou *et al*., 2007), produces antioxidant effect (Ghanbari *et* al., 2012). The extract of *Olea europaea L* provides defensive process by reducing the DOX cardiotoxicity which was articulated by the changes in peripheral and intracellular markers. It also diminished DOX provoked Inducible nitric oxide synthase (iNOS) induction, protein carbonyls content, lipid peroxidation and nitrotyrosine concentration in myocardial tissue in a dose dependent manner (Andreadou *et al*., 2007). The present study was conducted to evaluate the effects of extract on hematologic toxicosis associated with Doxorubicin when administered to rats.

Methods and materials

Study design

The study was designed in the Department of Pharmacology, Ziauddin University and conducted in Dow University of Health Sciences, following ethical and institutional approval. The research adhered to the ''Principles of Laboratory Animal Care'' (NIH publication #85-23, revised in 2011) and permitted by Board of Advance Studies and Research, Ziauddin University.

Preparation of extract

Freshly collected leaves 500 g were washed and air dried. Then by using standard grinding machine the leaves were grinded to fine powder. Before extraction process, the powder was macerated for 48 hours in 90% Ethanol. After maceration the extraction was performed two times. During each extraction approximately 1 liter of

Ethanol was used. Extraction was performed by using standard soxhlet apparatus. Then by utilizing standard rotary evaporator the final filtrate was concentrated to dryness under reduced pressure and stored for dosing at 4 ^{o}C.

Chemical used

DMSO (Dimethyl sulfoxide) and water for injection were used as a vehicle in administration of extract by Intra Peritoneal route. Water for injection was also used as a vehicle in administration of Doxorubicin by Intra Peritoneal route. The chemotherapeutic agent i.e. Doxorubicin was purchased from Time medicos Karachi and the leaves were obtained from market.

Statistical analysis

Data was analyzed on SPSS version 19 with paired sample test, p value < 0.05 was considered significant, p value < 0.01 highly significant and p value < 0.001 was considered very highly significant.

Animal protocols

Healthy male albino Wistar rats (weight 220-250 g) were purchased from AKUHS. The temperature was maintained at 23 ± 2 ºC. Relative Humidity was kept 65-75%. Light and dark cycle of 10:14 h. Standard polypropylene cages with wire mesh tops were use to keep the animals. Food and tap water was provided *ad libitum*. The animals were adapted to the constant environment for a period of seven days with sufficient rat chow, before the dosing started.

Dosing protocol

Eighteen animals were incorporated in the study. The animals were separated further in three groups based on treatments.

First group was control group labeled as **T*** [2 ml (0.9% Normal Saline)]

Second group was Toxic group labeled as **TP0** [(Doxorubicin) 15mg/kg]

Third group was treated group labeled as **TP1** [(extract of *Olea europaea L* + DXR) (200 + 15 mg/kg)].

The drugs were administered by IP (intraperitoneal) route. The injections were carefully made at the midway of the xyphoid and the pelvic bone (lower right quadrant of the abdomen, close to the midline). Needle (25 needle gauge) was used to administer the doses. The doses were administered on 7 days x 8 weeks. The blood was sampled by cardiac puncture on day 57th during which the animal was kept deeply anesthetized by using chloroform.

Biochemical assessment

Blood drawn by cardiac puncture was collected in anticoagulant tubes. For estimation of hematological parameters, blood (2 mL) was collected in EDTA K3 tubes for examination of Red Blood Cells, Hemoglobin, Leucocytes, Platelets, Neutrophils, Lymphocytes, Eosinophils, Monocytes (Newland, 2007) on automatic Humacount plus (3 part differential with histogram). Automated Hematology analyzer Model # 16400/S, (Human Germany).

Results

Results were categorized in terms of mean using Table and figures. They demonstrate difference in the levels of CBC and platelet count of rats among treated group TP1 (extract of *Olea europaea L*) and the toxic group TP0 (Doxorubicin). The Hemoglobin levels of animals were not significantly increased i.e. (p-value > 0.05), the levels of Red blood cells were increased significantly i.e. (p-value < 0.05) in Table 1 and Fig. 1 and 2, levels of Leucocyte were highly significantly increased (p-value < 0.01) and Neutrophil were highly significantly increased i.e. (p-value < 0.01) as shown in Fig. 3, level of Lymphocytes were highly significantly increased i.e. (p-value < 0.01) as shown in Fig. 5, levels of Eosinophil and Monocyte were not significantly increased i.e. (p-value > 0.05) as shown in Fig. 6 and the levels of Platelets were highly significantly increased i.e. (p-value < 0.01) as shown in Fig. 4.

Discussion

The parameters on which hematological toxicity of Doxorubicin was assessed were onset and severity of anemia, thrombocytopenia, leukocytopenia, neutropenia and lymphopenia. The administration of Doxorubicin had been linked with dose related decrease in the levels of hematologic parameters (Arancia *et al*., 1988). The levels of Hemoglobin and RBCs are considerably decreased as compared to the control group. This decrease in number of cells can be linked with the possibility of anemia in the animal due to the destruction of RBCs.

Table-I. Comparative Hematological profile of TP1 (*Olea europaea L* + Doxorubicin) with Control (TP0) (Doxorubicin)

Hematologic Profile	Groups	t-value	Degree of freedom	p-value
Hemoglobin (g/dl)	TP0 – TP1	-2.2665	5	0.0728****
RBC (x 10^6/mm^3)	TP0 – TP1	-3.7963	5	0.0127*
Leucocytes (x 10^3/mm^3)	TP0 – TP1	-5.0000	5	0.0041**
Platelets (x 10^3/mL)	TP0 – TP1	5.1235	5	0.0037**
Neutrophils (x 10^3/mm^3)	TP0 – TP1	-4.4177	5	0.0069**
Lymphocytes (x 10^3/mm^3)	TP0 – TP1	4.8166	5	0.0017**
Eosinophils (x 10^3/mm^3)	TP0 – TP1	-2.0761	5	0.0925****
Monocytes (x 10^3/mm^3)	TP0 – TP1	-2.059	5	0.0946****

*p-value < 0.05 (Significant), ** p-value < 0.01 (highly Significant), ***p-value < 0.001 (very highly Significant), **** p-value > 0.05 (Not Significant)

Arancia *et al.* (1988) stated in their study that Doxorubicin can cause non-regenerative anemia by causing toxic effects on erythrocyte precursors in the bone marrow or may cause regenerative anemia which is associated with increased destruction of poikilocyte (Arancia *et al*., 1988). The proper mechanism by which DOX alters RBCs morphology is not known. Gonsalvez *et al.* (1979) suggested that the potential mechanisms for poikilocytosis generation consist of development of reactive oxygen species which causes lipid peroxidation of Na^+/K^+ dependent ATP activity (Gonsalvez *et al*., 1979). The administration of extract eradicates the possibility of anemia by significantly increasing the levels of Hemoglobin and RBCs in the rats.

Mild Leukocytopenia is also observed in the rats receiving DOX. This is due to the immunosuppressive potential of Doxorubicin. The levels of Leukocyte are elevated after the administration of extract which can be observed in Fig.3. Results shows the Neutrophil levels are drastically decreased which is a positive sign for Neutropenia. Neutropenia is the most common abnormality of Doxorubicin. A drastic reduction in the levels of Thrombocytes can also be observed in Fig.4, which indicated thrombocytopenia but it is not associated with any type of bleeding. The levels of Lymphocytes, Eosinophils and Monocytes are also decreased due to the administration of DOX as compared to control group. The administration of extract increases all the levels of Platelets, Neutrophils, Lymphocytes, Eosinophils and Monocytes in a significant manner as shown in Fig. 4, 5 and 6. The effects of extract can imitate a rebound thrombocytosis which results in the stimulation of bone marrow due to thrombocytopenic or neutropenic episodes. Thrombopoietin was release as a result of thrombocytopenia which motivates production of platelets in the bone marrow (McDonald, 1987). Neutropenia causes the release of Granulocyte-macrophage colony-stimulating factor which in response stimulates the cell line which enhances the production of granulocytes and agranulocytes. This indicates that the extract strengthens the immune system by improving the cell count (Lee-Huang *et al*., 2004).

Conclusion

The study established that extract of *Olea europaea L* produces characteristic preventive effects on Doxorubicin induced hematologic toxicosis in rats by elevating the cell count.

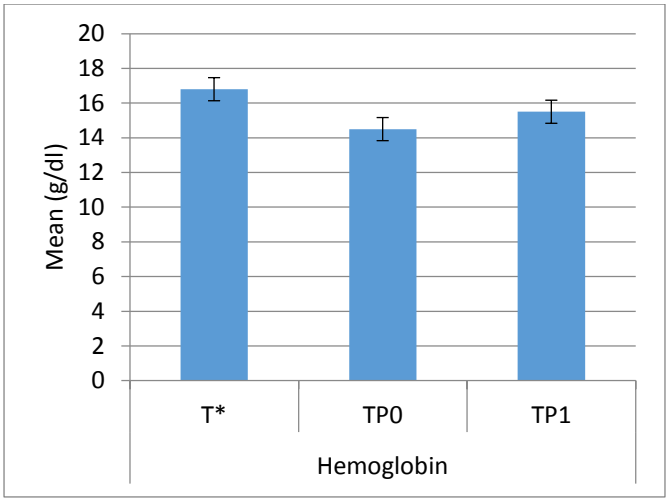

Figure-1. Hemoglobin levels in different groups.

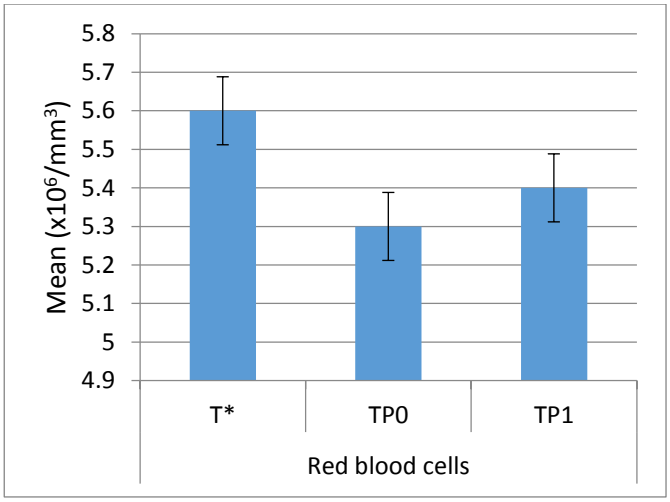

Figure-2. RBC levels in different groups.

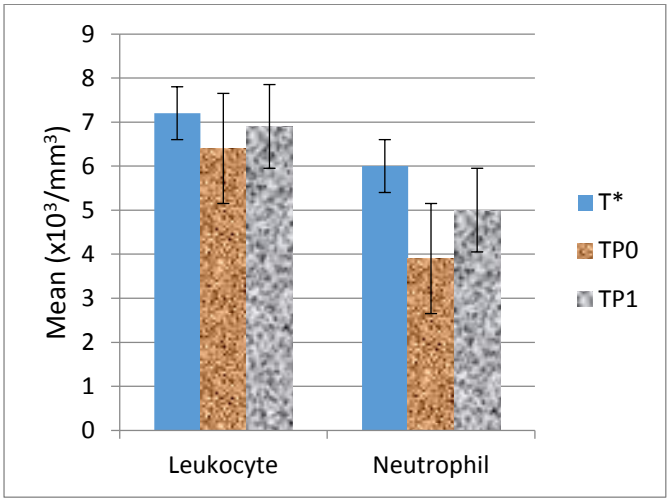

Figure-3. Comparative profile of Hematological profile in different groups.

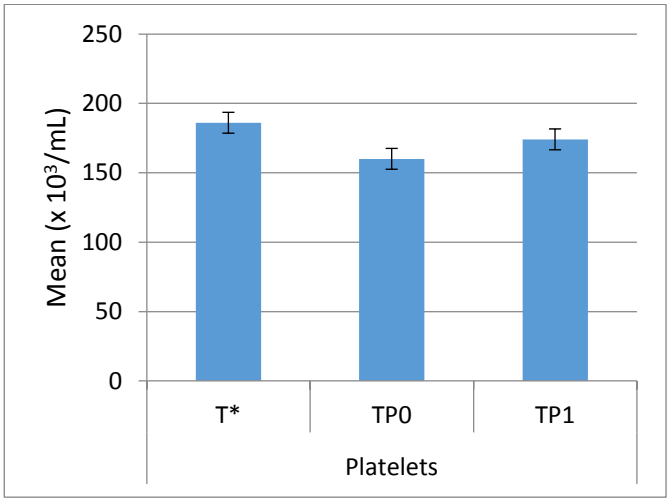

Figure-4. Platelet levels in different groups.

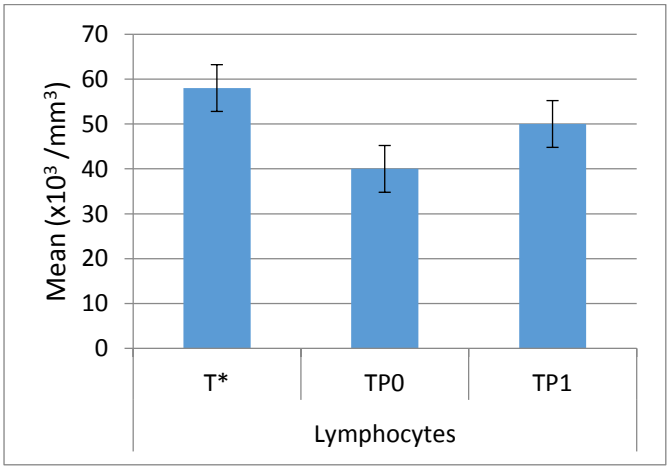

Figure-5. Lymphocyte levels in different groups.

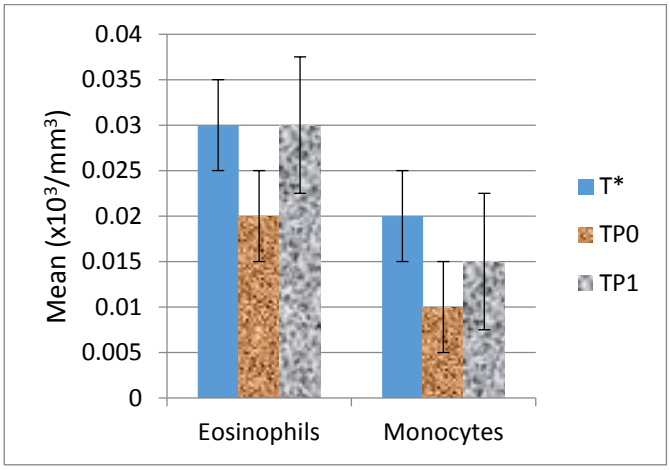

Figure-6. Comparative levels of Hematological profile in different groups.

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