



## HEMATOLOGIC TOXICOSIS; INDUCED BY DOXORUBICIN PROTECTIVE EFFECTS OF COMBINATION OF OLEA EUROPEAE AND GINKGO BILOBA

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**ABSTRACT... Objectives:** To evaluate the protective effects of combination of extracts of *Olea europaea* and *Ginkgo biloba* in response to hematological toxicosis induced by DOX. **Design of study:** Animal experimental study. **Setting:** Dow University of Health Sciences. **Period:** 2 month. **Materials & methods:** The extracts of *Olea europaea* and *Ginkgo biloba* were prepared by using standard Soxhlet apparatus and then they were mixed together in 1:1 ratio. Eighteen Male Wistar rats were incorporated in the study and divided in 3 groups: T\* (control group: 2 ml 0.9 % NS), TP0 (DOX group: 15 mg/kg/week) and TP1 (extract of *Olea europaea* & *Ginkgo biloba* + DOX: 200 + 15 mg/kg/week). All animals receive drugs by intra-peritoneal route. On 57<sup>th</sup> day blood was withdrawn by cardiac puncture and preserved for analysis. Hematological profile includes CBC and Platelet count. **Results:** Hematological analysis reveals that the animals which receive Doxorubicin had low levels of Hemoglobin, RBCs, Leukocytes, Neutrophils, Eosinophils, Monocytes, Lymphocytes and Platelets when compared to control group. The levels of Hb and RBCs were low but it is not evident of anemia. Neutrophil and Platelet were drastically low, which shows presence of Neutropenia and Thrombocytopenia. The treated group animals had shown positive elevation in the levels of CBC and Platelet count. **Conclusions:** On the basis of hematological report it is concluded that combination of extracts of *Olea europaea* and *Ginkgo biloba* can prevent the toxicosis effects of Doxorubicin.

**Key words:** Doxorubicin *Ginkgo biloba* Hematological profile *Olea europaea* Toxicosis

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## INTRODUCTION

In 1950's Anthracycline (first agent DAUNORUBICIN) from *Streptomyces peucetius* (soil bacterium) was derived.<sup>1</sup> Later on derivative of Daunorubicin was identified i.e. Doxorubicin, which was more effective anti-tumor agent.<sup>2</sup> DOX was a potent and highly effective drug in treating many varieties of cancer. Due to its toxic effects on heart, its usage was controlled.<sup>3</sup> The potential mechanisms of cardiac toxicity were free radical production, lipid peroxidation<sup>3</sup>, destruction of mitochondrial assembly<sup>4</sup>, release of vasoactive amine<sup>5</sup> and cellular toxicity. It also reacts with iron to initiate the production of ROS which causes cell death by apoptosis.<sup>5</sup>

Olive (*Olea europaea*) belongs to Oleaceae family had a long history for its medicinal, nutritional and health benefit effects. Phenolic constituents including oleuropein, hydroxytyrosol

and tyrosol were responsible for most of the olive pharmacologic activities.<sup>6</sup> The extract in a time and dose dependent manner have decreased proliferation and possess cytotoxic effects,<sup>7</sup> also inhibits production of ROS, decrease in the production of Thromboxane A2, anti-inflammatory and inhibition of platelet aggregation,<sup>8</sup> reduces infarct size,<sup>9</sup> produces antioxidant effect.<sup>6</sup> The extract of *Olea europaea* put forth a defensive process by reducing the DOX cardiotoxicity which was articulated by the changes in peripheral and intracellular markers. It also diminished DOX provoked iNOS induction, protein carbonyls content, lipid peroxidation and nitrotyrosine concentration in myocardial tissue in a dose dependent manner.<sup>9</sup>

*Ginkgo biloba* tree, member of Ginkgoaceae family was recognized about 170 million years ago.<sup>10</sup> The constituents which were present in

Ginkgo biloba leaves were flavonol glycosides, phenolic acids, terpene trilactones, polyphenols, biflavones, alkylphenols, and proanthocyanidins.<sup>11</sup> In all of these the two major groups were flavones glycosides having 24% fraction and the other group is terpene trilactones having 6% fraction producing positive biological effects credited to Ginkgo biloba.<sup>11</sup> Mechanism of actions include: decreasing the release of NO, modulation of receptor densities and concentration of neurotransmitters<sup>12</sup>, antagonism of PAF, free radical scavenging, anti-oxidant activities<sup>13</sup> and inhibition of mitochondrial dysfunction. The extract decreases the aggregation of thrombocytes by enhancing synthesis of prostacyclins; also decreases proliferation of vascular smooth cells, enhances vessel function and prevents restenosis after angioplasty.<sup>14</sup> Also enhances the fluidity of RBCs, improves blood flow and also reduces viscosity of entire blood.<sup>15</sup> The current study was performed to assess the outcome of combination of extracts on hematologic toxicosis associated with Doxorubicin when administered to rats.

## METHODS & MATERIALS

The study was designed in the Department of Pharmacology, Ziauddin University and conducted in DUHS 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.

Healthy male albino Wistar rats (weight 220-250gms) were purchased from AKUHS. The temperature was maintained at  $23 \pm 2$  °C. Relative Humidity was kept 65-75%. Light and dark cycle of 10:14 hrs. Standard polypropylene cages with wire mesh tops were used 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.

Freshly collected leaves of *Olea europaea* and *Ginkgo biloba* in a quantity of five hundred grams each 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 for 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. Both extracts were then mixed in a ratio of 1:1 at the time of administration. DMSO (Dimethyl sulfoxide) and water for injection were used as a vehicle in administration of extract by IP route. Water for injection was also used as a vehicle in administration of Doxorubicin by IP route.

Eighteen animals were incorporated in the study. Male albino whistar rats were separated further in three groups (six in each group). First group was control group labeled as T\* [received 2 ml (0.9%) NS], second group was Standard group labeled as TP0 [received Doxorubicin 15mg/kg/week] and treated group was labeled as TP1 [prophylactically received extract of *Olea europaea* & *Ginkgo biloba* 200 mg/kg/day followed by DXR 15 mg/kg/week]. The drugs were administered IP (intraperitoneal). 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 days 1,2,3,4,5,6,7 x 8 weeks. The blood was sampled by cardiac puncture on day 57<sup>th</sup> during which the animal was kept deeply anesthetized by using chloroform.

Blood drawn by cardiac puncture was collected in anticoagulant tubes. For estimation of hematological parameters, blood (2 ml) will be collected in EDTA K3 tubes for examination of Red Blood Cells, Hemoglobin, Leucocytes, Platelets, Neutrophils, Lymphocytes, Eosinophils, Monocytes<sup>16</sup> on automatic Humacount plus (3 part differential with histogram). Automated Hematology analyzer Model # 16400/S, (Human Germany).

## 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.

**RESULTS**

Results demonstrated variation in the levels of CBC and platelet count of rats in treated group TP1 (extract of *Olea europaea* and *Ginkgo biloba*) compared with the Standard group TP0 (Doxorubicin). Table-I and Figure-1 shows Hemoglobin levels were highly significantly enhanced (p-value < 0.01) compared with the standard. Whereas levels of Red blood cells count were also highly significantly increased (p-value < 0.01) compared with the standard as shown in Figure-2. When compared with the standard levels of Leucocyte and Neutrophil count were very highly significantly enhanced (p-value < 0.001) as shown in Figure-3, level of Lymphocytes were very highly drastically increased (p-value < 0.001) as shown in Figure-5 and levels of Eosinophil and Monocyte were not considerably increased (p-value > 0.05) as shown in Figure-6. Levels of Platelets were very highly extensively improved (p-value < 0.001) as shown in Figure-4.

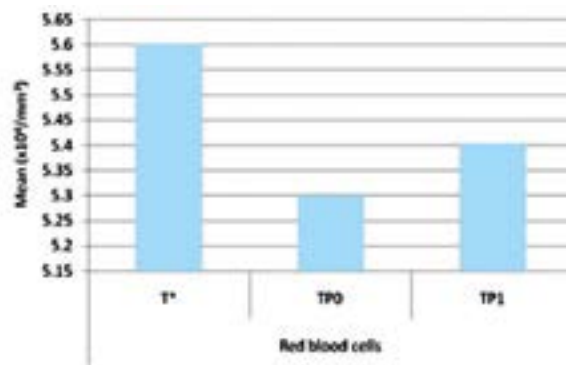


Figure-2. RBC levels of different groups

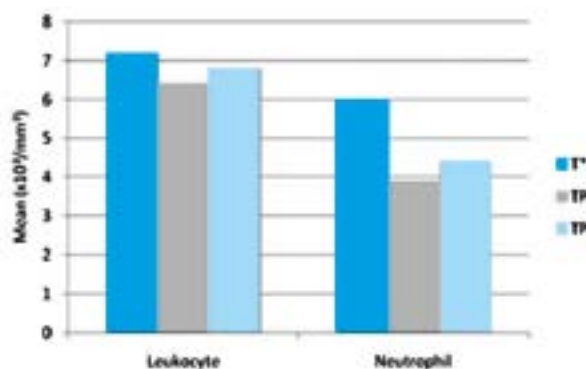


Figure-3. Comparative profile of Hematological profile of different groups

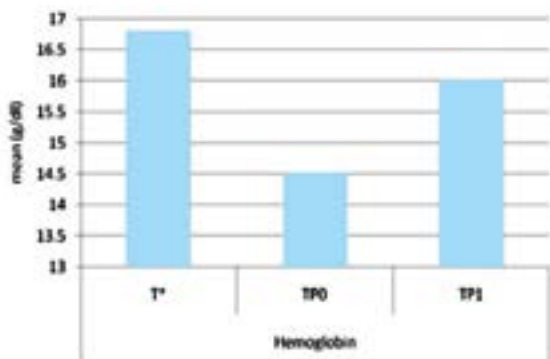


Figure-1. Hemoglobin levels of different groups

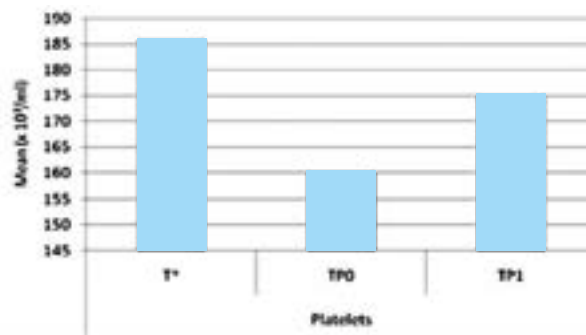


Figure-4. Platelet levels of different groups

| Hematologic Profile                               | Groups    | t-value  | Degree of freedom | p-value    |
|---|-----------|----------|-------------------|------------|
| Hemoglobin (g/dl)                                 | TP0 – TP1 | 6.6058   | 5                 | 0.0012**   |
| RBC (x 10 <sup>6</sup> /mm <sup>3</sup> )         | TP0 – TP1 | 4.3916   | 5                 | 0.0071**   |
| Leucocytes (x 10 <sup>3</sup> /mm <sup>3</sup> )  | TP0 – TP1 | 29.1471  | 5                 | 0.000***   |
| Platelets (x 10 <sup>3</sup> /mL)                 | TP0 – TP1 | -20.9969 | 5                 | 0.000***   |
| Neutrophils (x 10 <sup>3</sup> /mm <sup>3</sup> ) | TP0 – TP1 | 17.9799  | 5                 | 0.000***   |
| Lymphocytes (x 10 <sup>3</sup> /mm <sup>3</sup> ) | TP0 – TP1 | -9.5507  | 5                 | 0.0002***  |
| Eosinophils (x 10 <sup>3</sup> /mm <sup>3</sup> ) | TP0 – TP1 | -0.6670  | 5                 | 0.5343**** |
| Monocytes (x 10 <sup>3</sup> /mm <sup>3</sup> )   | TP0 – TP1 | -2.0585  | 5                 | 0.0946**** |

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

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

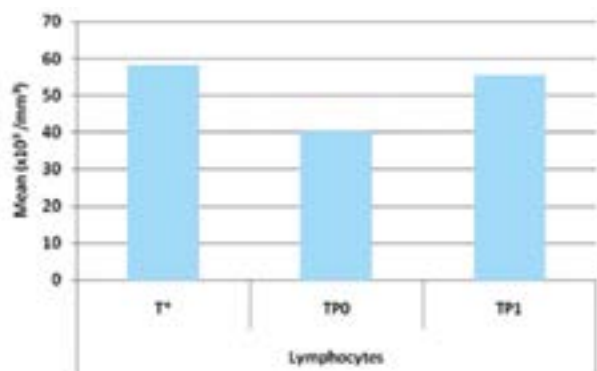


Figure-5. Lymphocyte levels of different groups

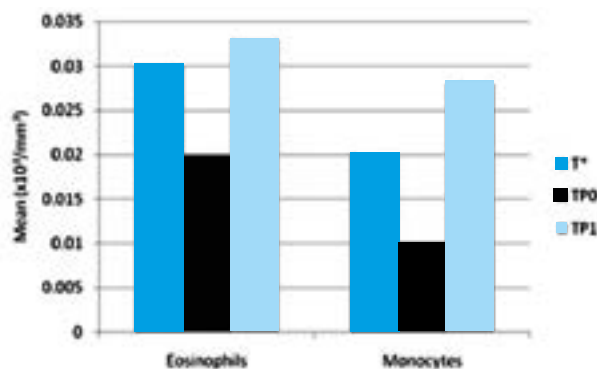


Figure-6. Comparative levels of Hematological profile of different groups

## DISCUSSION

Hematological toxicity was assessed by 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.<sup>17</sup> The levels of Hemoglobin and RBCs in standard group were 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. Arancia G, et al 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.<sup>17</sup> The proper mechanism by which DOX alters RBCs morphology is not known. Gonsalvez M, et al suggested that the potential mechanisms for poikilocytosis generation consist of development of reactive oxygen species which

causes lipid peroxidation of Na<sup>+</sup>/K<sup>+</sup> dependent ATP activity.<sup>18</sup> The administration of combination of extract eradicates the possibility of anemia by significantly increasing the levels of Hemoglobin and RBCs in the rats of treated group.

Mild Leukocytopenia was also observed in the rats receiving DOX. This results due to the immunosuppressive potential of Doxorubicin. The levels of Leukocyte were elevated after the administration of combination of extracts as shown in Figure-3. The group received Doxorubicin (standard group) shows the Neutrophil levels were drastically decrease which was an evident of Neutropenia. Neutropenia and thrombocytopenia was the most common adverse effect of Doxorubicin treatment. Drastic reductions in the levels of Thrombocytes indicate thrombocytopenia but it was not associated with any type of bleeding in the group treated with doxorubicin (standard group). The levels of Lymphocytes, Eosinophils and Monocytes were also decreased due to the administration of DOX in standard group as compared to control group. The prophylactic administration of combination of extracts (treated group) showed increase in the levels of Platelets, Neutrophils, Lymphocytes, Eosinophils and Monocytes count in a significant manner compared with the standard as shown in Figures-4,5 and 6. This indicates that the prophylactic treatment with the combination of extracts was effective in reducing Thrombocytopenia and Neutropenia associated with Doxorubicin therapy. The effects of extract imitate a rebound thrombocytosis which results in the stimulation of bone marrow due to thrombocytopenic or neutropenic episodes.<sup>19</sup> The combination of extract may mimic the natural mechanism by which Neutropenia causes the release of Granulocyte-macrophage colony-stimulating factor which in response stimulate the cell line which enhances the production of granulocytes and agranulocytes. This indicate that the combination strengthen the immune system by improving the cell count.

## CONCLUSION

The study established that Prophylactic treatment

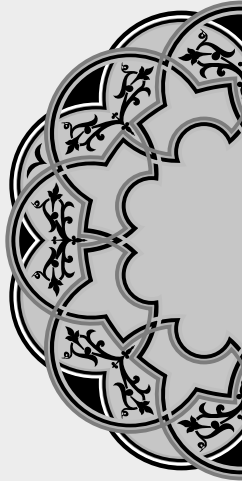
with combination of extracts of *Olea europaea* and *Ginkgo biloba* produces characteristic preventive effects on Doxorubicin induced hematologic toxicosis in rats.

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*“Expect the best,  
Prepare for the worst.”*

**Muhammad Ali Jinnah**

#### **AUTHORSHIP AND CONTRIBUTION DECLARATION**

| <b>Sr. #</b> | <b>Author-s Full Name</b> | <b>Contribution to the paper</b>   | <b>Author=s Signature</b> |
|--------------|---------------------------|--|---------------------------|
| 1            | Dr. Talha Bin Fayyaz      | Conception and designing of work, interpretation of data, drafting and final approval of manuscript, perform experimental work.              |                           |
| 2            | Prof. Zahida Memon        | Interpretation of data and give her expert view for manuscript designing. Giver research opinion and experience in finalizing the manuscript |                           |
| 3            | Dr. Mirza Tasawer Baig    | Help in procurement of herbs, preparation of extracts, and drafting.   |                           |
| 4            | Dr. Noor ul Ain           | Contributed in conception, Help in experimental work, data collection and manuscript writing.  |                           |
| 5            | Dr. M. Saddam Aziz        | Help in drafting and performing experimental work. Procurement of Drug.  |                           |