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**CAMPTOTHECIN VS. DOXORUBICIN: COMPARATIVE STUDIES BETWEEN  
NATURAL (CAMPTOTHECIN) AND SYNTHETIC (DOXORUBICIN)  
ANTICANCER AGENTS ON RED BLOOD CELLS**

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**ABSTRACT**

Camptothecin and doxorubicin are two eminent anticancer agents. Camptothecin and its derivatives are isolated from the plant sources and doxorubicin is a synthetic anticancer drug. Both are known for significant activity against many kind of cancers like breast cancer, cervical cancer and ovarian cancer. Although both of are proved to be very active against many cancers but still contain many side-effects. Camptothecin derivatives and doxorubicin induce the haemolytic anaemia in the patients. The present study was carried out to compare haemolytic effect of both the drugs on normal erythrocytes. Not only the haemolytic effect of these drugs were studied but also the protective effect of well-known antioxidants in presence of these drug was also studied using osmotic fragility assay. We not only evaluated the oxidative stress of selected anticancer agents but also the protective role of ascorbic acid,  $\beta$  Carotene, quercetin and tocopherol in camptothecin and doxorubicin treated red blood cells. The results showed that the doxorubicin causes more damage to RBCs than camptothecin at same concentration and condition. The results of protective effect of selected antioxidants showed that ascorbic acid and beta carotene were not able to protect the RBCs by oxidative damage but quercetin and tocopherol had moderate and significant protective effect on red blood cells caused by both the drugs.

**Keywords: Camptothecin, Doxorubicin, Osmotic Fragility, Antioxidants, Toxicity**

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## INTRODUCTION

After cardiovascular disease, cancer is the second leading causes of death worldwide. Millions of new cases are reported every year around the world. In 2019, approximately 1,762,450 new cancer cases and 606,880 cancer deaths were recorded in the United States only [1]. In KSA, cancer related deaths are increasing every year. According to Saudi Cancer Registry 2014, between January 01 and December 31 2014, the total number of newly diagnosed cancer cases reported in Saudi Arabia were 15,807. Total of 12,007 cases were reported among Saudi nationals, 3,640 among non-Saudi, and 160 of unknown nationality in 2018 [2]. There are many treatment options are available for the treatment of cancer but chemotherapy is the major solution for almost all kind of cancer. W.H.O approved many anticancer drugs. Although chemotherapy is one of the best option for cancer treatment but still is has many adverse effects on body. Anticancer drugs cause the major side-effect on blood most commonly anemia which as antagonistic impact on quality of life of the patient. Drug-induced haemolysis pathological condition which causes the breakdown of red blood cells results into severe anemia [3]. The drug-induced haemolysis is either immune or non-immune in origin [4]. The non-immune related haemolysis is the condition in which the drug of concern

causes lysis of red blood cells by damaging membrane integrity. That can involve direct effects on specific ion transport pathways by drugs, drug-induced oxidative damage of the cell membrane induced by the drug, and other adverse effects of the drug on cell volume control processes leads to the cell swelling results into cell membrane rupture [5, 6].

Approximately 50% cancer patients got anemia, and from them almost 20% of patients receiving chemotherapy need blood transfusion because their hemoglobin concentrations decline below 10 g/Dl [7]. The cycles of chemotherapy can result into erythropoiesis. There are two kind of anticancer drugs available on the basis of their origin; the naturally isolated drugs and the chemically synthesized drugs.

Natural are widely used for the treatment of many diseases. There are more 120 natural drugs which showed very promising anticancer activity with less side effects, although there are many synthetic drugs which showed good activity but many of them are reported to have severe side effects [8].

The present study was conducted to compare the haemolytic effect of natural anticancer agent camptothecin and synthetic anticancer drug doxorubicin in presence and absence of antioxidants.

Antioxidants play significant role in scavenging free radicals from the blood and other cells.

As the anticancer drugs are directly administered intravenously in the blood so concentration of the drug present in blood is higher than the other organs which can cause major oxidative stress in the blood cells. The administration of antioxidants along with the chemotherapeutic agents can exert a positive effect and can neutralize the negative effect [9, 10]. The present study was conducted to investigate the effect of two anticancer agents; one obtained from natural sources (Camptothecin) and other synthetically derived anticancer agent (Doxorubicin) on human blood cells and the protective role of antioxidants (ascorbic acid,  $\beta$  Carotene, quercetin and tocopherol) on camptothecin and doxorubicin treated red blood cells *in vitro*.

#### **MATERIALS AND METHODS:**

Pure Camptothecin, doxorubicin, ascorbic acid,  $\beta$  Carotene, quercetin and vitamin E were purchased from Sigma Aldrich, USA. NaCl,  $\text{Na}_2\text{PO}_4$ , and  $\text{NaH}_2\text{PO}_4$  were brought from Panreac Quimica. Dimethyl sulphoxide (DMSO) ordered from Sisco Research Laboratories Pvt Ltd. Different concentrations of camptothecin, doxorubicin, ascorbic acid,  $\beta$  Carotene, quercetin and tocopherol was prepared by dissolving them in DMSO. The phosphate-buffer

saline was prepared by using NaCl,  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$  in autoclaved double distilled water.

#### **Blood sampling:**

Five millilitre of the blood was collected from healthy volunteers with their informed consent. All the experiments were carried out in duplicate, and the mean value of them was used in calculations.

#### **Treatment conditions:**

To study the osmotic fragility of erythrocytes in the presence of anticancer drugs camptothecin/doxorubicin and antioxidants (ascorbic acid,  $\beta$  Carotene, quercetin and tocopherol) three different conditions were used in present study. These conditions are as follows: Pre-treatment condition (blood was treated with antioxidants for 30 min prior to the treatment of anticancer drugs). Co-treatment (Antioxidants and anticancer drugs were added together) and post-treatment (anticancer drugs were added before antioxidants)

#### **Osmotic fragility assay :**

To study the osmotic haemolysis of erythrocytes due to camptothecin and doxorubicin, osmotic fragility assay was conducted. The method used is as follows; the heparinized blood was treated with the drugs (camptothecin and doxorubicin separately) and incubated separately at  $37^\circ\text{C}$  for 30 min. Saline solutions aliquots of different

concentrations were prepared (10 to 1 g/L). The erythrocytes treated with drugs were then transferred to the tubes containing decreasing concentrations of saline solution. After mixing carefully, the cell suspensions were left to equilibrate for 30 min and then the tubes were centrifuged at 3000 r/min for 5 min. Supernatant was collected after centrifugation and the absorbance was recorded at 540 nm, standardized against a blank solution containing 10 g/L saline supernatant (corresponds to 0% haemolysis). The recorded optical density (OD) of the supernatants indicates the degree of haemolysis of the erythrocytes caused by the drugs. The lysis percentage was calculated by dividing the Optical density of the supernatant obtained from a particular saline concentration by the OD of the standard (1 g/L) representing 100% haemolysis. Osmotic fragility curves were constructed by plotting the lysis percentage against the concentration of saline solutions. The MEF25, MEF50 and MEF75 (mean erythrocyte fragility) values, which are the saline concentrations at which 25%, 50% and 75%, respectively, red blood cells haemolysis (at standard pH and temperature) were calculated [11].

#### STATISTICAL ANALYSIS:

The mean values and plus/minus standard error were calculated. Statistical analysis was analysed with Student's t-test and

multiple regression analysis  $p < 005$  was measured as statistically significant

#### RESULTS:

The osmotic fragility of the red blood cells in the presence of camptothecin and doxorubicin alone or in blood sample pre-treated with ascorbic acid,  $\beta$  Carotene, quercetin and tocopherol was measured by measuring haemolysis in saline solution. The different concentrations of saline solution responsible for the 25%, 50% and 75% red blood cells lysis were evaluated and titled as MEF25, MEF50 and MEF75, respectively. The control values were  $0.5820 \pm 0.002305$ ,  $0.4375 \pm 0.00125$  and  $0.3000 \pm 0.002404$  respectively. The erythrocytes were also treated alone with Camptothecin and Doxorubicin as well and the results were compared (**Figure 1**)

MEF25, MEF50 and MEF75 for the pre-treatment condition and co-treatment conditions were tabulated. The results indicating that the pre-treatment of ascorbic acid and beta carotene didn't have any significant role in protection of camptothecin or doxorubicin treated cells from oxidative damage but quercetin has only moderate protective effect on camptothecin or doxorubicin treated erythrocytes. Tocopherol treated caused the significance protection of red blood cells from oxidative damage in both the natural as well as synthetic anticancer agents (**Figure 2 & 3**).

In co-treatment and post-treatment (antioxidants were added with drugs and after exposure of anticancer drugs for 30

min), there was no change on the damage caused by anticancer drugs (Data not shown here).

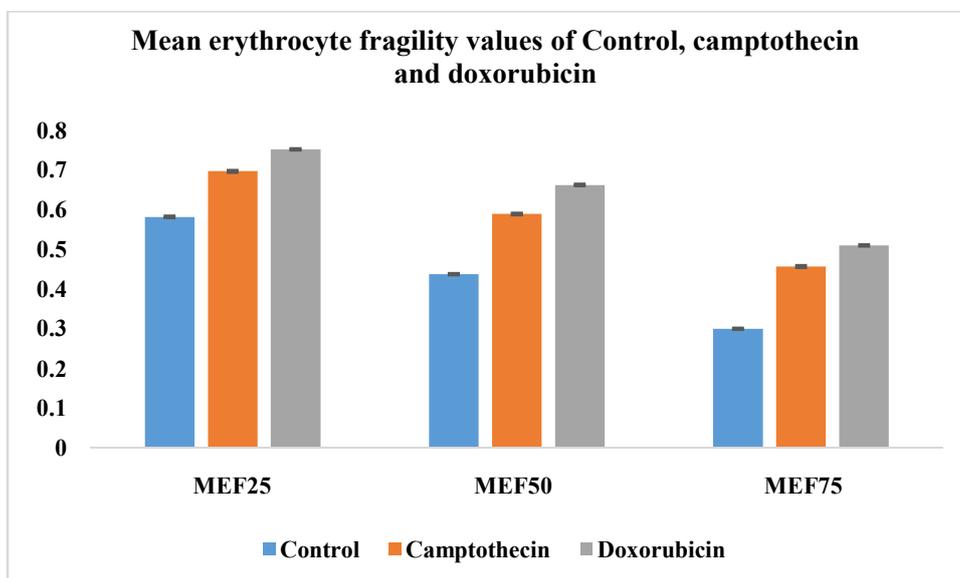


Figure 1: Comparison between control, camptothecin (1 ml/ml) and doxorubicin (1 ml/ml), resulting in mean erythrocyte fragility (MEF)

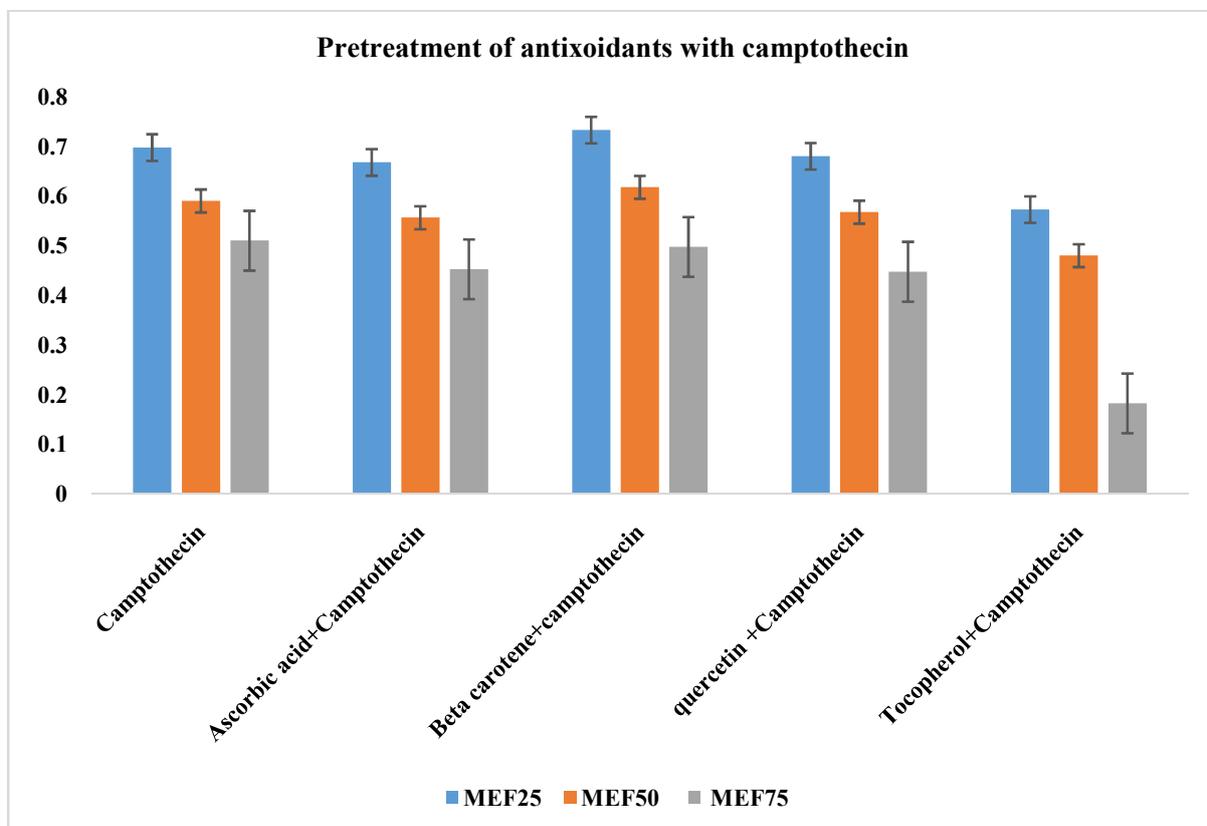


Figure 2: Pre-treatment of Ascorbic acid,  $\beta$  Carotene, Quercetin, and tocopherol (1 ml/ml) with camptothecin (1 ml/ml), resulting in mean erythrocyte fragility (MEF)

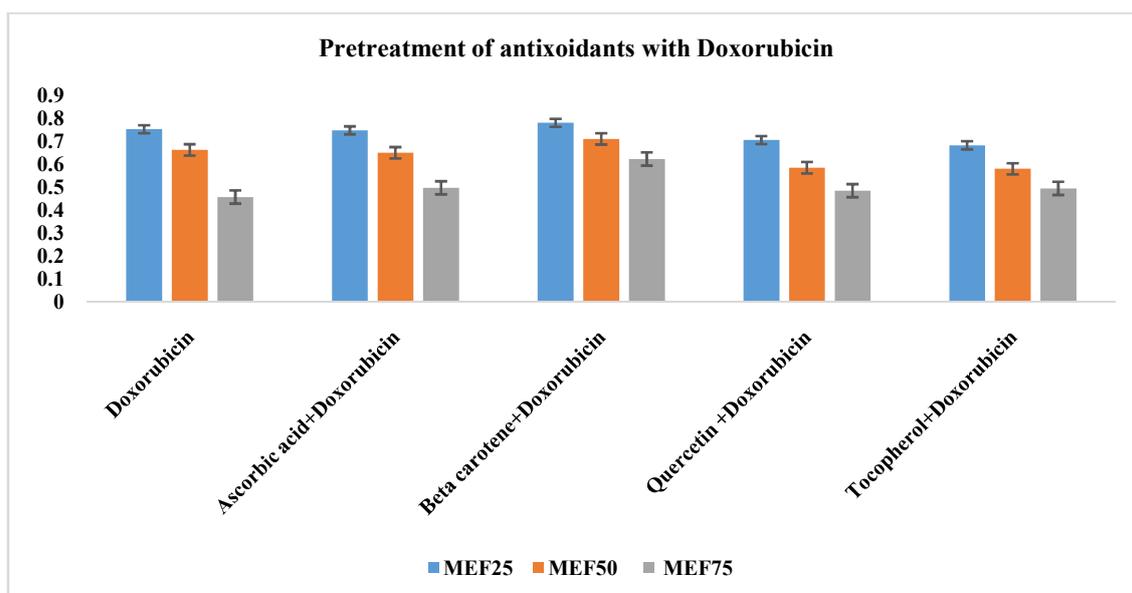


Figure 3: Pre- treatment of Ascorbic acid,  $\beta$  Carotene, Quercetin, and tocopherol (1 ml/ml) with doxorubicin (1 ml/ml), resulting in mean erythrocyte fragility (MEF)

## DISCUSSION:

Drug-induced haematological damage is one of the very common side-effects of many anticancer drugs as these are directly admixture in the blood and high concentration of these drugs in blood causes the severe oxidative stress on blood cells [12]. The result can be the low immunity due to the loss of white blood cells and severe anaemia due to osmotic lysis of the red blood cells [13]. The acute haemolytic anaemia caused by the anticancer drugs is one of the reasons when the cancer patients undergoing chemotherapy, haemoglobin drops sharply below 10 g/L, results in the blood transfusion [14, 15].

In several reports, it is shown that many chemotherapeutic agents induce haemolysis in human as well as in primates especially the chemically synthesised drugs [16].

There is always competition between natural and synthetic drugs. Although more than fifty percent anticancer drugs are isolated from the natural resources but still there are many synthetically derived anticancer drugs like doxorubicin are also used. The studies indicated that the drugs from natural sources carry less side effects in compare to the synthetic one especially in cancer patients who have weakened immune systems [17, 18]. The present study was conducted to prove the low toxicity of natural drug camptothecin in comparison with the synthetic drug doxorubicin on the red blood cells. The present study was not only to compare the toxicity of natural and synthetic anticancer agents but also the role of antioxidants to reduce the damage caused by those drugs.

The antioxidants are well known to lower the oxidative damage in the living

system[19]. The osmotic fragility test results indicated that the doxorubicin caused little higher damage in comparison with the camptothecin when both the drugs were added in blood sample alone. In other set of experiments the blood was treated with four well known antioxidants ascorbic acid and beta carotene, quercetin, tocopherol before adding the anticancer agents. The result of pre-treatment of antioxidants shows that Ascorbic acid and beta carotene didn't exert any positive effect on blood cells but quercetin had moderate protection. Tocopherol showed significant protection of red blood cells caused by both the drugs.

#### CONCLUSION:

The present study suggested that the use of drugs isolated from natural resources may have less side effects than the synthetic drugs and the use of antioxidants can minimize the damage caused by anticancer agents in patients.

#### DECLARATION OF CONFLICTING INTERESTS:

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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