

# Immune potentiating and antitoxic effects of camel milk against cyclophosphamide-induced toxicity in BALB/C mice

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## ABSTRACT

**Objective:** Cyclophosphamide (CYP), a cytotoxic anticancer drug, causes a substantial reduction in leukocytes numbers, called leukopenia. Leukopenia is a major predisposing factor to infections caused by opportunistic pathogens. The aim of the present study is to assess the effects of camel milk consumption against CYP-induced leukopenia and other toxic effects.

**Methods:** CYP at a dose of 250 mg/kg was injected in the mice through the intraperitoneal route. Each mouse was orally administered with 1 ml of camel milk twice daily for 10 days. The blood was taken from various groups of mice to determine quantitative and qualitative changes in the leukocytes. The protective role of camel milk against CYP-induced toxicity was also assessed by determining the levels of antioxidant enzymes, superoxide dismutase (SOD), and catalase (CAT) in the hepatic tissue homogenates.

**Results:** Mice injected with CYP showed substantial weight loss and a simultaneous depletion of leukocytes. Oral administration of camel milk protected mice against CYP-induced toxicity. The group of CYP-injected mice that received camel milk showed lesser decrease in their weight and leukocyte numbers. CYP-injected mice showed lower levels of SOD and CAT, whereas simultaneous consumption of camel milk resulted in lesser decrease in the levels of SOD and CAT in liver homogenates.

**Conclusions:** The results of the present study suggest that camel milk may have an immunopotentiating role in diseases or conditions associated with leukopenia- or drug-induced toxicities.

**Keywords:** Camel milk, cyclophosphamide, leukopenia, toxicity

## Introduction

Leukopenia, reduced numbers of circulating white blood cells (WBC), may result from reduced production or increased utilization and destruction of the WBC because of certain infections, drugs, malignancy, megaloblastosis, and hypersplenism.<sup>1-3</sup> Viral infections will often cause the body to slow bone marrow function and thus also lower the WBC count.<sup>2</sup> Infections or autoimmune disorders that kill off cells faster than the production will intensify this reaction.<sup>4</sup> Congenital disorders or cancerous growths can also cause the body to slow its bone marrow function.

Chemotherapy or radiation therapy or the long-term use of antibiotics can kill the WBC as the medications target fast-growing tissues throughout the body.<sup>5</sup> Cyclophosphamide (CYP), an anticancer drug, alkylates DNA and protein by producing cross-links.<sup>6</sup> CYP causes leukopenia due to damage of bone marrow progenitor cells. It causes immune

suppression in the treated patients. Patients undergoing CYP chemotherapy become easy targets of opportunistic pathogens due to reduced numbers or function of the WBC.<sup>7-9</sup> Antioxidant supplementation can influence the response to chemotherapy and alleviate the development of adverse side effects resulting from chemotherapy.<sup>10</sup> Natural compounds that reduce the side effects, as well as stimulate the immunity, may be of great help in improving cancer treatment strategies.<sup>11</sup>

Camel milk, a very good source of proteins, has immense biological effects associated with the improvement of infectious diseases.<sup>12,13</sup> Camel milk can reduce up to 35% of the insulin daily dose taken by type-1 diabetic patients, with a reduction in the anti-insulin antibodies.<sup>14-16</sup> Camel milk ingestion increases bone calcium, makes it effective in calcium metabolism disorders.<sup>17</sup> Camel milk has been reported to possess antiallergic property.<sup>18</sup> Camel milk liposomes loaded with anticancer agents, etoposide and doxorubicin have been shown to be effective against murine fibrosarcoma.<sup>19</sup> Camel

milk shows the antigenotoxic and anticytotoxic effects in cisplatin-treated mice.<sup>20</sup> Keeping into consideration of many beneficial effects of camel milk, we are investigating the protective role of camel milk consumption against CYP-induced leukopenia and other toxic effects.

## Materials and Methods

CYP was purchased from Tocris Biosciences (Bristol, UK). The WBC counting fluid (Turk's fluid) and Giemsa stain were purchased from Loba Chemie (Mumbai, India). Fresh camel milk was obtained from camel farm at Al-Bukayriyah, Qassim, Saudi Arabia. Superoxide dismutase (SOD) and catalase (CAT) estimation kits were purchased from Biovision Inc. (CA, USA).

## Mice

Female BALB/C mice of 8-10 weeks age were used in the study. Mice were obtained from the animal house facility of King Saud University, Riyadh, Saudi Arabia. The techniques used for bleeding and injection, as well as the sacrifice of mice, were approved by the Animal Ethics Committee of the College of Applied Medical Sciences, Qassim University, Buraydah, Saudi Arabia.

## Induction of leukopenia in mice

A single dose of CYP (250 mg/kg) was injected through intraperitoneal route to induce temporary leukopenia as described earlier.<sup>21</sup> Mice were divided into the following groups and each group contained 5 mice.

1. Control
2. Camel milk (CM)
3. CYP
4. CYP + Camel milk (CYP+CM).

## Quantitative and qualitative analysis of leukocytes

The numbers of leukocyte were counted on day 10 post-CYP treatment in both untreated and CM-treated mice. Blood, taken from mice, was mixed with WBCs dilution fluid (Turk's fluid), and the leukocytes were counted using a Neubauer chamber as described earlier.<sup>22</sup>

To assess the qualitative changes in leukocytes, a blood smear was made on the slide. Slides were fixed with methanol for 1 min and then stained with Giemsa reagent for 5 min. After staining, slides were washed and allowed to dry. The smear was observed under microscope to see the changes in the leukocytes.

## Monitoring of the weight loss in CYP-injected mice

The toxicity of CYP was assessed by monitoring the relative weight loss in CYP-injected mice or CYP-injected mice receiving camel milk as compared to normal mice.

## Biochemical analyses

The levels of antioxidant enzymes were determined as described earlier.<sup>23</sup> To determine the levels of hepatic antioxidant enzymes SOD and CAT, the liver tissue samples were rinsed in the cold phosphate-buffered saline (PBS), and the connective tissue was removed. The tissue samples were then homogenized in PBS and centrifuged at 5000 g for 15 min at 4°C to collect the supernatant fractions, which were then used to assay the enzyme activities using specific kits (Biovision Inc., Milpitas, CA, USA).

## Statistical analysis

Various groups were compared using One-way ANOVA using Prism software (Version 6.0, San Diego, USA).

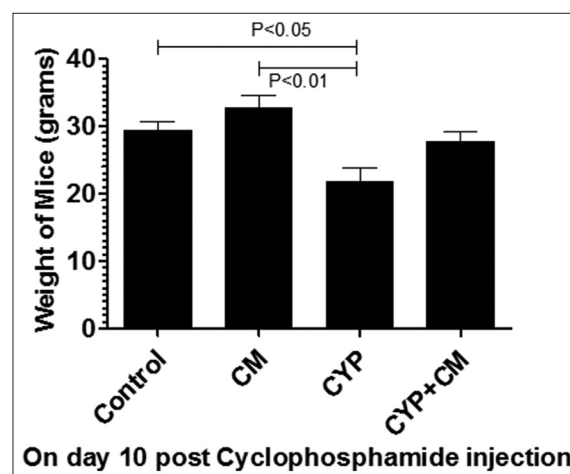
## Results

### Consumption of camel milk protects against CYP-induced weight loss

The administration of CYP causes behavioral changes in the mice. Mice were observed daily for their mortality, morbidity, and weight loss. The group of mice injected with CYP showed a significant weight loss (~21.8 g) as compared to mice from a normal control group (~29.4 g) (Figure 1). The group of CYP-injected mice that received camel milk showed less reduction in their body weight (~27.8 g) as compared to CYP-injected mice that did not receive camel milk (~21.8 g).

### Consumption of camel milk protects against CYP-induced leukopenia

The protective effect of camel milk against CYP-induced toxicity was determined by assessing the quantitative and qualitative changes in leukocytes. There was a substantial reduction of leukocytes in CYP-injected mice with mean



**Figure 1:** Administration of cyclophosphamide causes weight loss in the mice

leukocyte count  $\sim 2433$  as compared to the control group of mice with  $\sim 5640$  leukocytes per  $\text{mm}^3$  of the blood ( $P < 0.01$ ) whereas the group of mice injected with CYP followed by camel milk consumption has  $\sim 4019$  leukocytes per  $\text{mm}^3$  of the blood (Figure 2). The administration of camel milk increased the total number of leukocytes from  $5640/\text{mm}^3$  of the blood to  $7010/\text{mm}^3$  of the blood in normal controls (Figure 2).

### CYP induces toxic changes in leukocytes

The blood picture developed on day 10, post-CYP injection showed some structural changes in the leukocytes. Leukocytes from CYP-injected mice showed morphological changes, including disorganized nuclei, dispersed chromatin strands, and granules as compared to leukocytes from normal mice (Figure 3a and c). CYP toxicity was observed in all types of leukocytes, including lymphocytes, neutrophils, and monocytes. Neutrophils, a type of granulocytes, were the first cells to show the effects of CYP-induced toxicity in the form of degranulation, whereas lymphocytes mainly showed vacuolation and condensation of nuclei (Figure 3c). Mice received camel milk did not show any toxic changes in leukocytes (Figure 3b). Moreover, Cyclophosphamide-injected mice that received camel milk showed reduced manifestations in their leukocytes (Figure 3d)

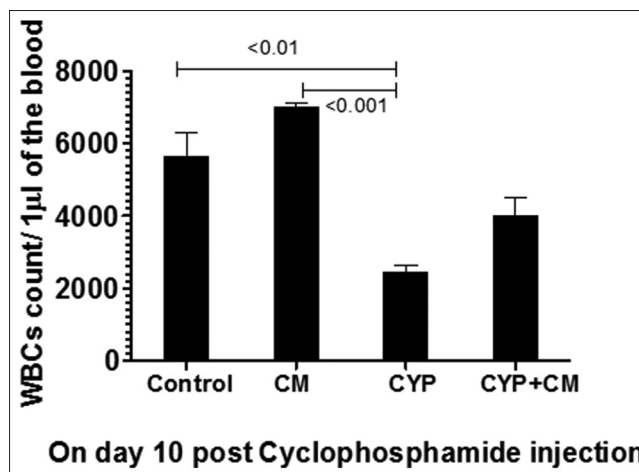
### Consumption of camel milk alleviates CYP-induced liver toxicity

The results of the present study showed that CYP significantly decreased the levels of hepatic SOD as compared to that of the control group ( $P < 0.001$ ). The consumption of camel milk recovered the activity of SOD against CYP-induced inhibition (Figure 4a). This recovery in the activity of SOD was found to be significant against CYP-induced inhibition of SOD activity ( $P < 0.001$ ).

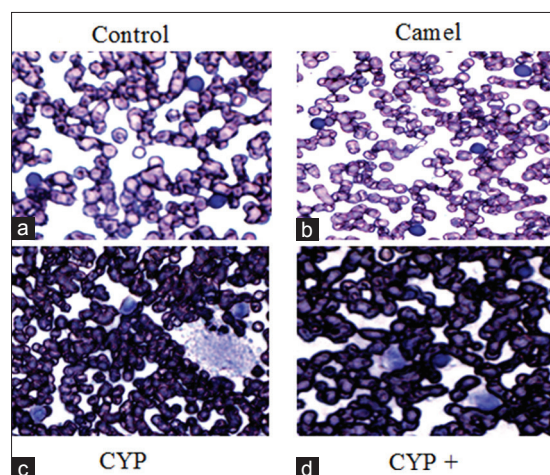
Like SOD activity, the enzymatic activity of hepatic CAT was also found to be significantly reduced in the CYP-injected mice as compared to its activity in normal mice (Figure 4b,  $P < 0.001$ ). The activity of CAT was found to be significantly higher in the CYP-injected mice that consumed camel milk as compared to the group of CYP-injected mice that did not consume the same ( $P < 0.05$ ).

## Discussion

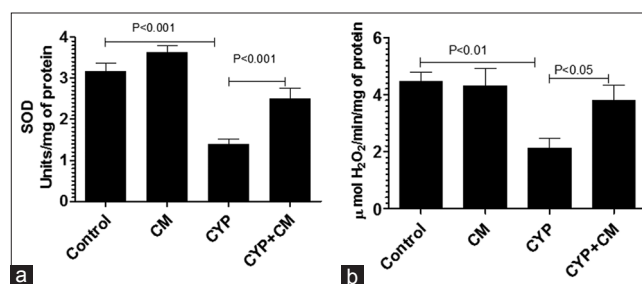
CYP has been used in the treatment of several types of cancers. Since it has immunosuppressive properties, it has also been used in the treatment of many autoimmune diseases, non-specific immunopathies, or in the prevention of organ rejection.<sup>24</sup> The major effect of CYP treatment is on leukocytes, including neutrophils and lymphocytes depending on the dose and timing of the drug administration.<sup>24,25</sup> Sometimes CYP-induced leukopenia



**Figure 2:** Effect of camel milk consumption against cyclophosphamide-induced leukopenia



**Figure 3:** Leukocytes from (a) normal control mice (b) mice received camel milk (c) cyclophosphamide (CYP)-injected mice (d) CYP-injected mice that consumed camel milk



**Figure 4:** The consumption of camel milk recovers the activities of SOD and CAT in CYP-injected mice

may result in life-threatening consequences. CYP is a cytotoxic chemotherapeutic drug acting as an alkylating agent. It produces reactive carbonium ion that reacts with DNA.<sup>26</sup> The initial activation reaction of CYP is carried out by microsomal oxidation system in liver producing 4-hydroxy CYP, which in turn acts as a cytotoxic metabolite and diffuses out of hepatocytes into plasma and distributed throughout the body. Then, 4-hydroxy CYP is further



converted into phosphoramidate mustard, acrolein, and other cytotoxic metabolites.<sup>27</sup> Phosphoramidate mustard is known to cause myelosuppression.

It has been shown that immunomodulatory compounds used with chemotherapy may reduce myelosuppression and thus enhance the immune response. We have earlier shown that immunomodulator tuftsin has the ability to reverse CYP-induced leukopenia in mice.<sup>21</sup> Tuftsin-pretreated leukopenic mice show more resistance to fungal infections compared to leukopenic mice not pretreated with tuftsin.<sup>21</sup> The present study aimed to determine whether the oral administration of camel milk can reduce the CYP-induced immunosuppression in mice. The results of the present study showed that CYP caused toxicity to the mice and also caused the depletion of leukocytes to a remarkably low level. The administration of camel milk opposed the toxic effects of CYP. When compared with the CYP-treated leukopenic mice, total leukocyte count increased in the group of mice orally administered with camel milk. The metabolism of CYP produces highly reactive free radicals, which cause toxicity to cells, including leukocytes.<sup>9</sup> The protective effect of camel milk may be attributed to its strong antioxidant property. SOD and CAT are important antioxidant enzymes, which protects the body cells from the oxidative damage by reactive oxygen species.<sup>23</sup> Our earlier report showed that thymoquinone protected the liver and reversed CYP-induced depletion of hepatic SOD and CAT.<sup>23</sup> Camel milk consumption has been reported to increase the levels of antioxidant enzymes.<sup>28</sup> Camel milk protein also improved the functioning of neutrophils in older rats.<sup>29</sup> Earlier studies have shown the antigenotoxic and an anticytotoxic effect of camel milk against cisplatin-induced toxicity in mice.<sup>20</sup> The results of the present study showed that the consumption of camel milk protected the activities of SOD and CAT in CYP-injected mice. These findings are in agreement with the studies that show the camel milk possesses antitoxic and immunopotentiating properties.

## Conclusions

The present study shows that camel milk has the ability to reverse the CYP-induced leukopenia and weight loss in mice. Moreover, it also helps in the recovery of important antioxidant enzymes such as SOD and CAT that are important players in the innate immune responses.

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