

## The Protective Effect of Pomegranate, *Punica granatum*, on Murine Malaria

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**Abstract.-** Malaria is still one of the most devastating infectious diseases in the tropics. The present study aims to investigate the protective role of pomegranate peel extract against *Plasmodium chabaudi*-induced spleen tissue damage in mice. Animals were divided into three groups. Group I served as a vehicle control. Group II and group III were infected with  $10^6$  *P. chabaudi*-infected erythrocytes. Group III was gavaged with 100  $\mu$ l of 300 mg/kg pomegranate peel extract for 6 days. All mice were sacrificed at day 6 post-infection. Treated mice with pomegranate significantly showed approximately 50% reduction in parasitemia compared to untreated control. Infection also induced a weight loss. Histochemical studies revealed that infection caused a decrease in both carbohydrates and protein contents in the spleen. Pomegranate could improve these altered changes. Based on these results, it is concluded that pomegranate peel could offer protection against splenic tissue damage.

**Key words:** Malaria, pomegranate peel, spleen.

### INTRODUCTION

Malaria, which is caused by the apicomplexan parasite *Plasmodium*, is a major cause of morbidity and mortality throughout the world particularly in developing countries (Mehlhorn, 2001). Young children and pregnant women are the groups most affected. It has been estimated that half the world's population are at risk of malaria transmission in more than 100 countries (WHO, 2012). This has urged on intensive drug discovery endeavors geared towards identifying novel, potent and cheap anti-malarial drugs. However, the identification of quality leads from natural sources would significantly augment these efforts.

Pomegranate (*Punica granatum*) has been used in the folk medicine of many cultures especially in the Middle East. It has been reported that pomegranate exhibits antiviral, antioxidant, antidiabetic, antidiarrheal, anticancer, anticomoidal and antiproliferative activities (Abdel Moneim,

2012; Abdel Moneim and El-Khadragy, 2013; Dkhil, 2013)

The spleen plays an important role (especially in areas where malaria is endemic) producing antibodies against the malarial parasites (Mokashi *et al.*, 1992). The normal function of the spleen is to remove abnormal erythrocytes and intraerythrocytic inclusions. Malaria-infected red blood cells (iRBCs) contain a highly proliferative *Plasmodium* parasite which undergoes different blood stages of asexual cycle (Sherman, 1998). Changes in splenic structure during the course of malaria can result in asymptomatic enlargement (Dkhil, 2009). However, there is no reported study demonstrates the effect of pomegranate on malaria induced infection in spleen. Therefore, the current study aims to investigate the antimalarial role of pomegranate peel extract (PPE) as well as examining its ameliorative role on *P. chabaudi* induced spleen damage in mice.

### MATERIALS AND METHODS

*Preparation of the pomegranate peel extract (PPE)*

*P. granatum* peels were obtained from pomegranate fruit purchased from a local market. The samples were authenticated by Dr. Jacob Thomas (Botany Department, College of Science,

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King Saud University, Saudi Arabia). PPE was prepared according to the method described by (Abdel Moneim, 2012) with some modification. Air dried powder (100 g) of pomegranate peels was extracted by percolation at room temperature with 70% methanol and kept at 4°C for 24 h. The obtained extract was concentrated under reduced pressure (bath temperature 50°C) and dried in a vacuum evaporator. The residue was dissolved in distilled water and used in this experiment.

### Animals

Twenty four Swiss albino mice were bred under specified pathogen-free conditions and fed a standard diet and water *ad libitum*. The experiments were performed only on mice at an age of 9-11 weeks and were approved by state authorities and followed Saudi Arabian rules for animal protection.

### Infection of mice

Blood stages of *Plasmodium chabaudi* were weekly passaged in Swiss albino mice. Blood levels of *Plasmodium* sp. were maintained in 9- to 14-week old Swiss albino mice (weight, 20-25 g) by weekly passages of infected blood as described previously (Wunderlich *et al.*, 1991). Experimental animals were challenged with  $10^6$  *P. chabaudi*-parasitized erythrocytes. Parasitemia was evaluated in Giemsa stained blood smears, and total erythrocytes were counted in a Neubauer chamber.

### Experimental design

Animals were divided into three groups. The first group served as a vehicle control. The second and the third group were infected with  $10^6$  *Plasmodium chabaudi*-parasitized erythrocytes. The third group was gavaged with 100 µl of 300 mg/kg PPE for 6 days (Dkhil, 2013). All mice were sacrificed at day 10 post-infection.

### Histochemistry of spleen

Pieces of spleen were formalin fixed at room temperature overnight, embedded in paraffin and 5 µm sections were prepared. Spleen sections were stained with periodic acid-Schiff's method to demonstrate total carbohydrates (Hotchkiss, 1948) and with bromophenol blue method to demonstrate total proteins (Mazia *et al.*, 1953). Prepared slides

were carefully examined for each animal and at least three slides from different areas of the organ were examined.

### Statistical analysis

Statistical analyses were performed using Student's *t*-test;  $p \leq 0.05$  is considered statistically significant.

## RESULTS

Through examination of blood smears (Fig.1), parasitemia were found to be evident on day 4 p.i., On day 5 p.i., the parasitemia reached about 18% and on day 6 p.i., it reached the maximum level (about 40%). PPE was able to reduce the parasitemia to about half on day 6 p.i. (Table I).

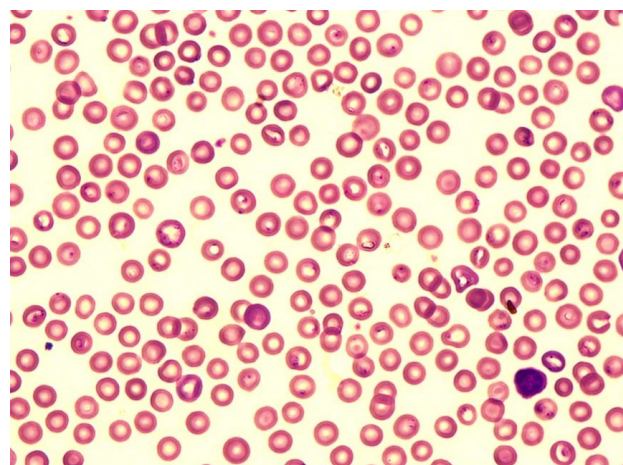


Fig. 1. Giemsa stained blood smear from mouse infected with *P. chabaudi*. Magnification  $\times 100$ .

**Table I-** Pomegranate induced changes in parasitemia

Days p.i.	Parasitemia (%)	
	Infected (- PPE)	Infected (+ PPE)
Day 4	1.6 $\pm$ 0.8	1.2 $\pm$ 0.4
Day 5	18 $\pm$ 4	13 $\pm$ 3*
Day 6	41 $\pm$ 6	23 $\pm$ 5*

Values are means  $\pm$  SD. \* indicates statistical significance at  $p < 0.05$  (Student *t* test) compared to untreated.

On day 6 p.i., the infection induced a significant weight loss (Fig. 2). Treatment of mice

with 300 mg/Kg PPE caused a significant increase in the reduced weight due to infection (Fig. 2).

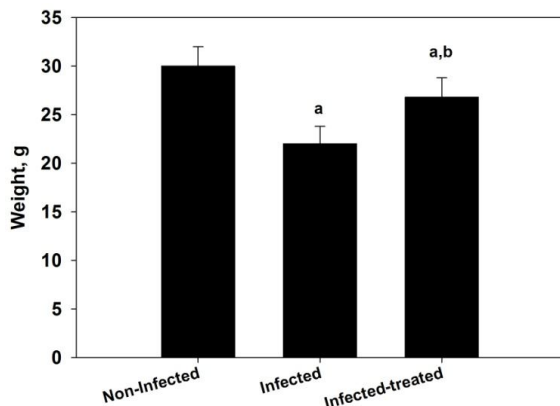


Fig. 2. Pomegranate induced changes in weight of *P. chabaudi* infected mice. Values are means  $\pm$  SD; a, significant change at  $p \leq 0.05$  compared to non-infected mice; b, Significant change at  $p \leq 0.05$  between treated and untreated infected mice.

*P. chabaudi* infection caused a disturbance in the carbohydrate and protein content in spleen of mice. Control spleen sections stained with PAS method are shown in Figure 3A. Splens of infected mice showed a slight decrease in the carbohydrate content (Fig. 3B). Splens of mice treated with PPE could improve the decreased amount of carbohydrates (Fig. 3C).

The examination of spleen sections from the control group stained by bromophenol blue method showed normal protein content (Fig. 4A). Protein content was moderately decreased in the splenic cells of mice infected with *P. chabaudi* (Fig. 4B). PPE could improve the decreased content of proteins in the splens infected with *P. chabaudi* (Fig. 4C).

## DISCUSSION

The plant kingdom is a source of a vast array of natural products that have been exploited as medicaments for a variety of disease conditions. Traditional medicines have been used to treat malaria for many years and are the source of the two main groups of modern antimalarial drugs (artemisinin and quinine derivatives). These

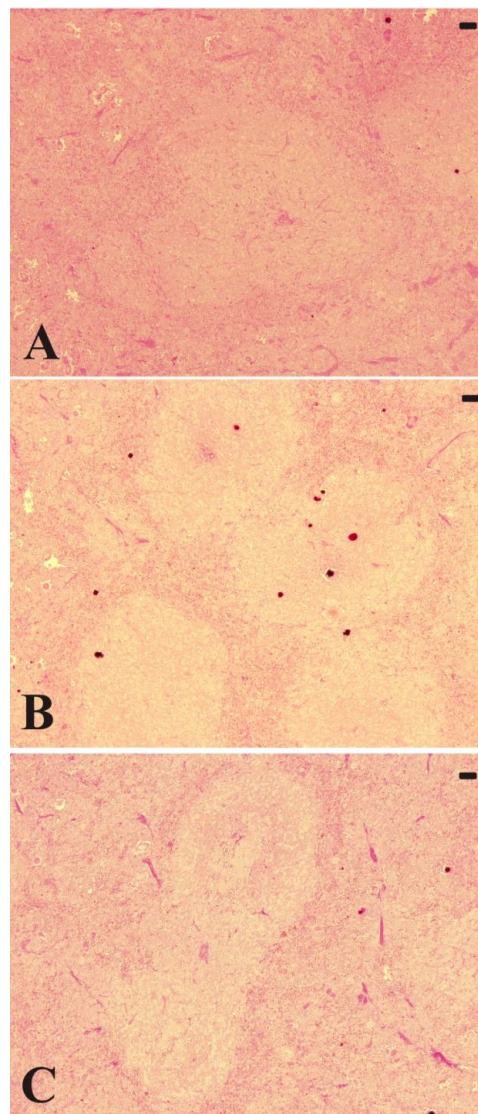


Fig. 3. Total carbohydrates in spleen sections; A, control spleen; B, spleen of infected mice with a slight decrease in carbohydrate content; C, spleen of infected pomegranate treated mice with some improvement. Sections were stained with periodic acid Schiff's method. Bar indicates 50  $\mu$ m.

antimalarial drugs were derived from plants and are still effective in treating malaria (Bodeker and Willcox, 2000) although they are relatively expensive for the rural dwellers that are predominantly at risk of the disease. Thus, such people resort to cheaper available traditional medicines which are major herbs.

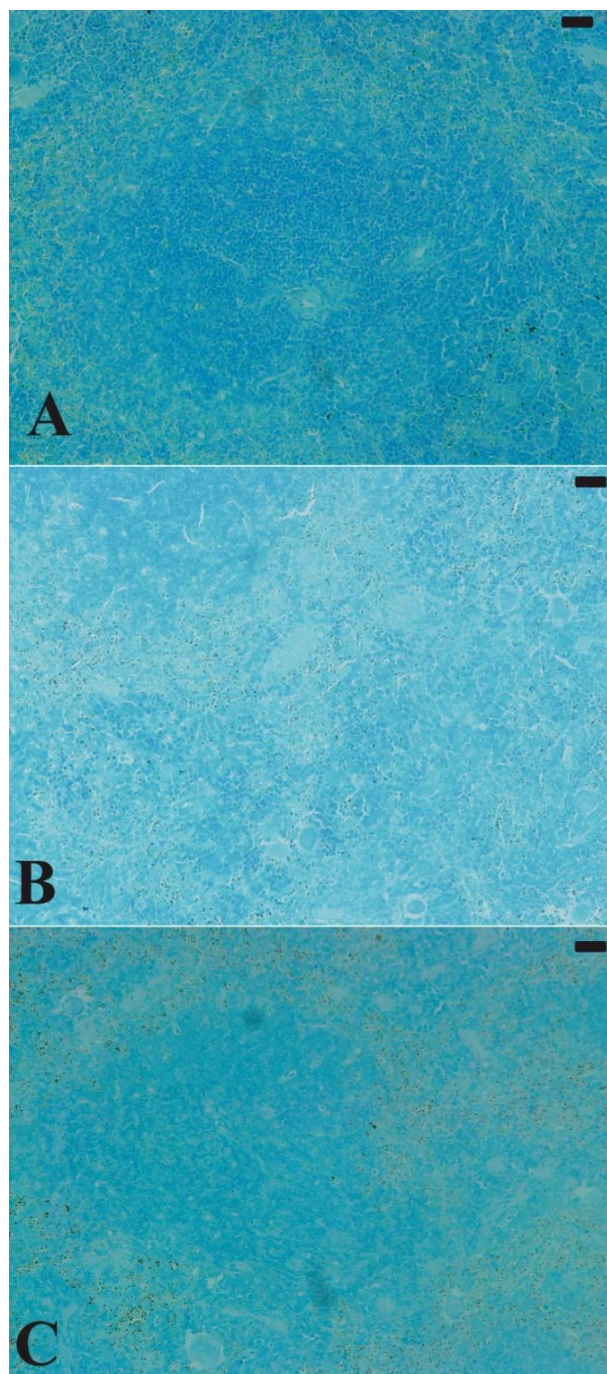


Fig. 4. Protein content in spleen sections; A, control spleen with a considerable amount of protein elements; B, infected spleen section with a weak response towards bromophenol blue reaction; C, spleen of infected-treated mice with some improvement. Sections were stained with bromophenol blue method. Bar indicates 50  $\mu$ m.

The spleen acts as an effectors against malaria infection (Chotivanich *et al.*, 2002). Normal spleen is composed of white and red pulps surrounded by a capsule of dense connective tissue. The white pulp is composed of a central, T-cell rich zone, and a periarterial lymphoid sheath surrounded by B-cell-rich primary follicles. The white pulp was separated from the red pulp by the marginal sinus embedded in a layer of marginal zone lymphocytes. During the course of *P. chabaudi* infection there is a disturbed T-cell areas and changes in splenic architecture (Dkhil, 2009).

Pomegranate has strong antioxidant and anti-inflammatory properties (Abdel Moneim, 2012). Despite the many studies conducted to examine the efficacy of *P. granatum* in treating many diseases and microbial infections, much remains unknown about its effects on parasitic infections. However, some studies have indicated that *P. granatum* has anti-helminthic (Fernandes *et al.*, 2005; Korayem *et al.*, 1993) and anti-protozoan activities (Calzada *et al.*, 2006; Dell'Agli *et al.*, 2010). Recently, Dkhil (2013) reported the anthelmintic and anticoccidial activity of the pomegranate peel extract. Furthermore, Dell'Agli *et al.* (2010) reported the antimalarial activity of the fruit of pomegranate in vitro.

The intraerythrocytic malaria parasites rely mainly on glycolysis for energy generation (Olszewski and Llinas, 2011). Glucose from the plasma enters into the *Plasmodium* cytoplasm and is subsequently degraded to lactate via the anaerobic Embden-Meyerhoff-Parnas (EMP) pathway (Crawford *et al.*, 2003). *P. falciparum* was shown to have a high activity of the glycolytic enzymes and of lactate dehydrogenase when compared to uninfected RBC (Roth *et al.*, 1988). This phenomena is consistent with our study which showed the evident reduction of total carbohydrate in *P. chabaudi* infected mice compared to that of uninfected indicating may be the homology between different malaria species in highly demanding of carbohydrate fermentation to meet the energy requirement for highly proliferative parasite. However, further biochemical and molecular studies need to be conducted to determine these similarities. Interestingly, PPE has demonstrated a protective effect on injured splenic tissue of PPE treated *P.*

*chabaudi* infected mice as compared to untreated.

Moreover, asexual malaria parasite blood stage obtains most of the amino acids required for protein synthesis and other metabolic functions from the digestion of RBC haemoglobin (Goldberg, 2005). However, haemoglobin is not the only source for amino acids but rather the *Plasmodium* salvage from the free amino acids pool in the plasma while some amino acids are biosynthesized by the parasite itself from glucose and CO<sub>2</sub> (Sherman, 1977, 1979). Our study showed that the total protein content of spleen in *P. chabaudi* infected mice had a markedly weak response to bromophenol blue compared to uninfected. Significantly, the total protein content of spleen in PPE treated *P. chabaudi* infected mice revealed a better improvement and response when compared to untreated infected mice. This may indicate that PPE interfere with the parasite amino acid uptake processes. Overall, it appears that PPE has a potential protective role against malarial infection which can be used in controlling the disease.

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