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Rutin ability to reduce hematological toxicity induced by cytarabine in mice (Preventive effects of rutin towards hematological toxicity of cytarabine)

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ABSTRACT

This study was designed to determine the preventive effects of rutin toward the hematological toxicity of the anticancerous drug, cytarabine, in vivo using Balb C mice. The analysis of blood showed that subcutaneous injection of 50 mg/kg of cytarabine during three consecutive days caused a significant myelosupression. Indeed, the number of red and white blood cells decreased significantly ($p<0.05$). Moreover, the amount of hemoglobin and the percentage of the hematocrit decreased remarkably ($p<0.05$). However, the intra peritoneal injection of rutin (100 mg/kg) during six consecutive days didn't exert any toxicological effects and the number of red and white blood cells, the amount of hemoglobin and the percentage of the hematocrit remained the same as in the control. The combination of rutin and cytarabine, where rutin was administered to mice three days before and three days during cytarabine treatment, protected blood cells from a veritable toxicity. In fact, the number of red cells, the amount of hemoglobin and the percentage of the hematocrit were significantly higher. However, the number of white blood cells was slightly protected. On the other hand, the treatment with cytarabine alone resulted in an elevation of body temperature in mice which reached 39°C. While, the temperature of the group treated with the combination of rutin and cytarabine remained normal and did not exceed 37.5°C. The ability of rutin to reduce hematological toxicity induced by cytarabine may be an important therapeutic factor in the treatment of cancer.

Keywords: Rutin, cytarabine, flavonoids, chemoprevention, toxicity, antipyretic effect.

INTRODUCTION

Humans consuming high fruit and vegetables diets, may ingest up to 1 g of flavonoids daily [1, 2]. Flavonoids have therapeutic effects on disease conditions caused by oxidative stress, such as coronary atherosclerosis, ischemic damage, aging processes and cancer [3, 4]. Quercetin, the major representative of the flavonol subclass of flavonoids, is present mostly in the form of glycosides like quercetin-3-O-rhamnosylglucoside or rutin [5, 6]. To date, it is reported that more than 70 plant species contain rutin (Figure 1) [7]. It is present mostly in the outer layer of ingested fruits, the outer leaves of vegetables, the leaves of unprocessed tea and in wine [5, 6]. Some biological and pharmacological effects have been attributed to this flavonol such as anti-inflammatory activity [8-10], antioxidant activity [11-14] and antitumor activity [15]. It has been reported that rutin protected stomach from ulcer by enhancement of the antioxidant activity of glutathione peroxidase and inhibition of lipoperoxidation [16]. Furthermore, rutin has a demonstrated activity as a venotonic agent and is present in several pharmaceutical

products [4]. A lot of studies have demonstrated the preventive activity of rutin in a variety of laboratory animal models including azoxymethane (AOM)-induced colonic tumorigenesis in mice and rats [17-19]. For that reason, the natural rutin is one of the attractive phytochemicals and it is considered as an important flavonoïds in pharmaceutical industry. Over 130 therapeutic medicinal preparations that have been registered as drugs worldwide are containing rutin in their formulations [7].

Cytarabine (Cytostar-U), isolated from the Caribbean sponge (*Cryptotheca crypta*) is currently used in routine treatment of patients with leukemia and lymphoma [20, 21]. Because cytarabine action interferes with normal nucleoside metabolism, a number of normal cell types particularly dependent on salvage of nucleosides, are extremely sensitive to cytarabine, these include the bone marrow and intestinal mucosa. In patients with leukemias and non-Hodgkins lymphoma treated with cytarabine, gastrointestinal and hematopoietic toxicities predominate and frequently limit dose escalation [22]. Thus, the combination of drugs is more effective than any single constituent in achieving chemopreventive and anti-cancer effects.

The aim of this study is to investigate the protective effects of rutin against the hematological toxicity and fever caused by cytarabine using Balb C mice.

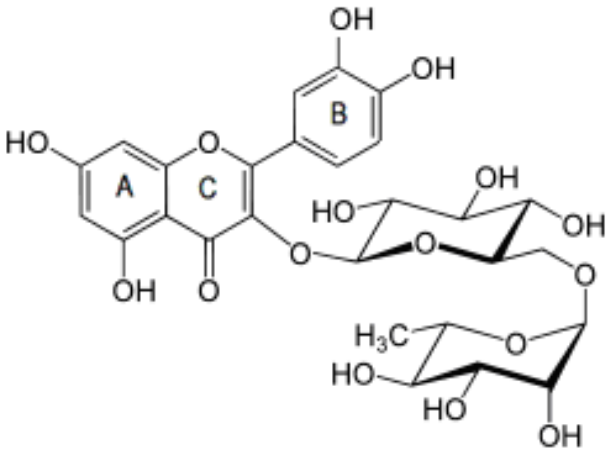

Figure 1: Chemical structure of rutin (quercetin-3-O-rhamnosyl glucoside).

MATERIALS AND METHODS

Chemicals

Rutin and carboxymethyl cellulose (CMC) were obtained from Sigma (Germany). Saline solution (NaCl, 0.9%) was obtained from BIOLYSE (Algeria) and Cytarabine was obtained from Pfizer.

Animals

Male and female Balb C mice weighting between 20 and 40 g were obtained from Pasteur Institute of Algiers, Algeria. They were housed in standard cages (48 cm × 35 cm × 22 cm) at room temperature (22 ± 2°C) for 12-h light/dark cycle. Mice were acclimatized at least for 1 week prior use with free access to food and water *ad libitum*. All procedures were performed in accordance with the European Union Guidelines for Animals Experimentation (2010/63/EU). Mice were divided into four groups each consisting of 5-8 mice. Two groups (control groups) received intra peritoneal injection doses of the vehicle carboxymethyl cellulose 1% during six days. From the 4^{th} to the 6^{th} day the first group was injected subcutaneously by cytarabine (50 mg/kg) and the second group was injected by the saline solution (NaCl 0.9 %). The 3^{rd} and 4^{th} groups, rutin and rutin combined with cytarabine (rutin/cytarabine) groups, received intra peritoneal injection doses of rutin 100 mg/kg during six days. From the 4^{th} to the 6^{th} day they were injected subcutaneously by saline solution or cytarabine (50 mg/kg) solution respectively. In the 7^{th} day, mice of whole groups were sacrificed by head dislocation and their bloods were collected in test tubes containing the anticoagulant EDTA.

Blood cell count
Bloods collected in test tubes containing the anticoagulant EDTA were analyzed automatically using a Coulter counter of blood cells (Beck Man coulter).

Body temperature measurement
Every day during the period of the experiments, the body temperature of mice of the whole groups was taken by ear measurement using an electronic thermometer (THERMOVAL ®).

Statistical analysis
The data are expressed as mean ± SEM (n=5-8 mice). A statistical analysis was performed using Student t test with the GraphPad INSTAT software system for Windows.

RESULTS AND DISCUSSION

The analysis of blood indicated that the subcutaneous injection of 50 mg/ml cytarabine caused a significant myelosuppression. Thus the number of red and white blood cells decreased significantly (Figure 2 and 3).

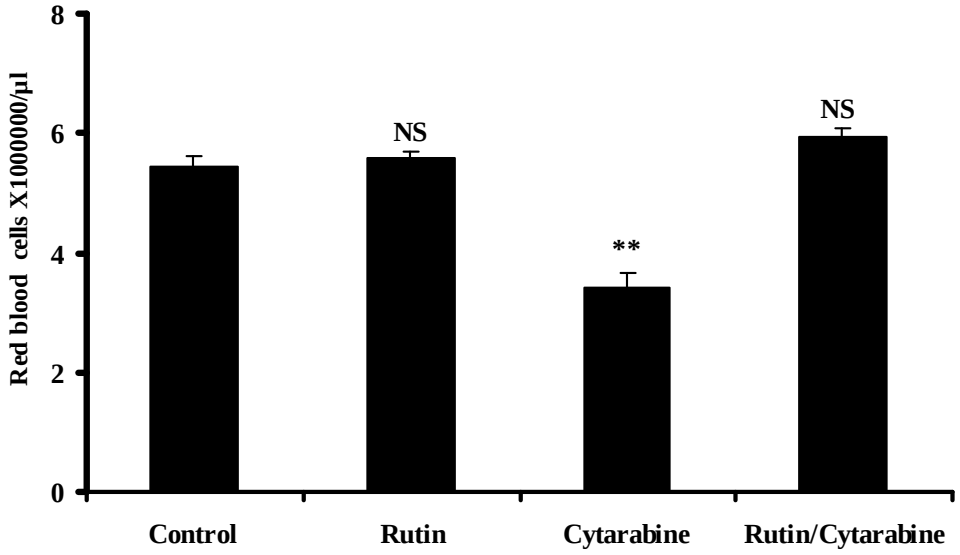

Figure 2: The toxicity of cytarabine (50 mg/kg) on red blood cells of mice Balb C and the protective effects of rutin (100 mg/kg). The results are expressed as means ± SEM. ** $P \leq 0.01$, NS: not significant Vs the control.

Furthermore, the amount of hemoglobin and the percentage of hematocrit decreased remarkably (table 1).

A lot of studies showed that the toxicity of cytarabine towards normal cells is due to its interference with the metabolism of nucleotides in cells with high turnover like bone marrow and intestinal mucosa cells [19].

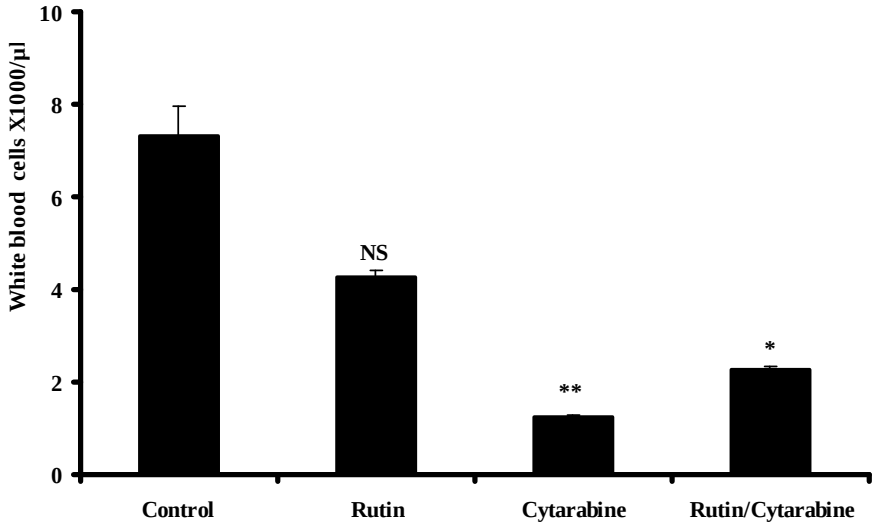

Figure 3: The toxicity of cytarabine (50 mg/kg) on white blood cells of mice Balb C and the protective effects of rutin (100 mg / kg). Results are expressed as means ± SEM. *$P \leq 0.05$, ** $P \leq 0.01$, NS: not significant Vs the control.

Table 1: The toxicity of cytarabine (50 mg/kg) on the amount of hemoglobin and the percentage of hematocrit and the protection effects of rutin (100 mg/kg). ** $P \leq 0.01$, NS: not significant Vs the control.

	Hemoglobin (g/dl)	Hematocrit (%)
Control	11.17 ± 0.60	31.15 ± 1.44
Rutin	9.93^{NS} ± 0.21	28.82^{NS} ± 0.53
Cytarabine	6.12** ± 0,33	19.90 **± 0.79
Rutin/Cytarabine	10.28^{NS} ± 0.25	29.56^{NS} ± 0.76

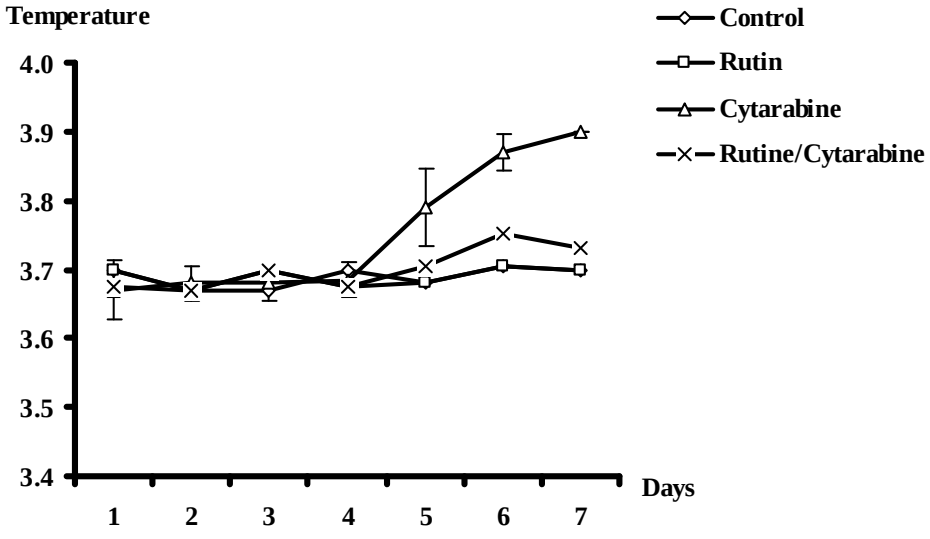

Figure 4: The antipyretic effect of rutin (100 mg/kg) on Balb C mice treated with subcutaneous injection of 50 mg/kg of cytarabine.

The intra peritoneal injection of 100 mg/kg of rutin during six consecutive days did not exert any cytotoxic effects and the blood cell number remained the same as in the control group (not treated group). Combinational treatment of

cytarabine with rutin (Rutin/Cytarabine) protected red blood cells from a veritable toxicity (Figure 2). However, white blood cells were slightly protected (Figure 3). The amount of hemoglobin and the percentage of hematocrit remained the same as the control (Table 1).

On the other hand, the results showed that cytarabine caused an elevation of body temperature which reached 39°C in the group treated with cytarabine alone. In contrast, the body temperature of the group treated with cytarabine combined with rutin remained normal and did not exceed 37.5°C (Figure 4).

This latter result could be explained by the anti-inflammatory effects of rutin. Indeed, it has been reported that rutin inhibited rat paw swelling induced by carrageenan and reduced neutrophil chemotaxis to formyl-Met-Leu-Phe (fMLP) [9]. In addition, rutin exerted a potent inhibitory effect on respiratory burst of fMLP-stimulated neutrophils [12], and inhibited histamine release and expression of proinflammatory cytokines in mast cells [20]. Furthermore, the administration of rutin at a dose of 25 mg/ kg body weight once daily for 21 days might have potential value in the treatment of rheumatoid arthritis [8].

There is overwhelming evidence from diverse studies that flavonoid glycosides, as well as their aglycones, exhibit significant biological activities such as antitumor, apoptotic, antimicrobial, and radical-scavenging activity, as well as immunoactivity coupled with low toxicity [25, 26]. Therefore, their use as potential therapeutic compounds against a variety of diseases is of great interest.

CONCLUSION

Numerous studies have reported the diverse pharmacological activities of rutin, as well as the risk reduction of diseases. The present study suggests that rutin may be of benefit against the side effects of cytarabine. Consequently, rutin appears to be a potential phytochemical ingredient in food supplement and medicinal products.

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