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Role of Rosemary Leaves Extract as A Protective Agent Against Azathioprine-Induced Toxicity in Rats

Hala M T El-Mougy*, Gehan A Youssef**
Medical Biochemistry*and Physiology** Departments,Faculty of Medicine (Girls) Al-Azhar University.

Abstract

Background: Rosemary is widely found along the coasts of the Mediterranean Sea. Its leaves or extract were found to have a high antioxidant and anti-inflammatory activity. It is also used as an antispasmodic, analgesic, anti-rheumatic and expectorant. These actions are mainly due to its content of essential oils. Azathioprine (AZA) is an immunosuppressive drug. It is widely used in many diseases. A major drawback is the occurrence of side-effects, especially acute pancreatitis.

Aim of the work: This work was done to study the effect of dietary supplement of rosemary leaves as a strategy for amelioration of the side-effects of azathioprine.

Material and Methods: Thirty-two adult male albino rats were used in this study. They were equally divided into four groups. Group I: control group, group II: rosemary group, the animals were given a daily oral dose of rosemary leaves extract. Group III: azathioprine group, the animals were given a single dose of AZA intraperitoneally. Group IV: rosemary azathioprine group: the rats were given daily doses of rosemary leaves extract then azathioprine in the last day of the experiment as in the previous regimen. The experiment continued for ten days. Blood samples were taken from all groups and examined for tumour necrosis factor alpha, serum amylase enzyme, C-reactive protein and renal function tests (serum urea and creatinine).

Results: Rosemary significantly decreased the levels of tumour necrosis factor alpha, serum amylase enzyme and serum urea and C-reactive protein in rosemary AZA group compared to AZA group .

Conclusion: The aqueous rosemary leaves extract has the ability to ameliorate the biochemical pathways of the side-effects of azathioprine, so it is advisable to give it concomitantly to patients treated by azathioprine.

Key words: azathioprine, rosemary, anti-inflammatory activity, antioxidant.

Introduction:

Rosemary leaves is a common household plant grow in many parts of the world as it is cheap and claimed to be safe. Its leaves possess a variety of bioactivities including antioxidants, anti-tumours, anti-inflammatory and anti-HIV. It contains a vast number of polyphenolics including carnosic acid, carnosol, rosemarinic acid and ursolic acid ***(Peng et al., 2007).***

Rosemary and its constituents, especially caffeic acid derivatives such as rosmarinic acid, have a therapeutic potential in treatment and prevention of bronchial asthma, spasmogenic disorders, peptic ulcers, inflammatory diseases, hepatotoxicity ***(Gutierrez et al., 2010).***

The effectiveness of rosemary extracts as antioxidants have caused their commercial use. It has a powerful inhibitory action on lipid peroxidation production and, a stimulatory action on the synthesis of cellular antioxidants ***(Ahmed and Abdalla, 2010).***

The activity of rosemary has been ascribed to the diterpene content, mainly carnosic acid and carnosol ***(Wijeratne and Cuppett, 2007)***, as well as to the essential oil constituents ***(Bozin et al., 2007).*** Carnosic acid provides protection from the liver carcinogen aflatoxin A ***(Costa et al., 2007)***.

Azathioprine is a myelotoxic and hepatotoxic immunosuppressive agent. Bone marrow and liver are the main targets but gastrointestinal tract, kidney, lungs, CNS and skin may also be affected. Transient gastroenteritis may be observed with massive overdose. Leukopenia is the main toxic effect which may occur during azathioprine therapy and in the overdose patient. Liver and kidney function tests may be altered but usually returned to normal after discontinuation of the drug ***(de Boer et al., 2005)***.

Azathioprine [6-(1-methyl-4-nitro-5-imidazolyl) thiopurine], is a common immunosuppressant that has been used in the treatment of hematological malignancies, inflammatory bowel disease and autoimmune conditions such as rheumatoid arthritis and following transplantation to avoid organ rejection. However, AZA use has been complicated by a high incidence of serious adverse drug reactions including hepatotoxicity in rats and elevation of reactive oxygen species leading to mitochondrial injury and cell death due to necrosis ***(Shanmugarajan and Devaki, 2008).***

TNF- α (cachectin) is a member of a group of cytokines that stimulates the acute phase reaction. It is produced by activated macrophages, T and B cells, natural killer cells, astrocytes, endothelial cells, smooth muscle cells and some tumour cells. It plays critical roles in normal host resistance to infection and to the growth of malignant tumours, serving as immune-stimulants and as mediators of the inflammatory response. Over-production of TNF-α, however, has been implicated as playing a role in a number of pathological conditions, including cachexia, septic shock, and autoimmune disorders ***(Locksley et al., 2001)***.

C-reactive protein (CRP) is a member of the class of acute-phase reactants synthesized by the liver, its levels rise dramatically during inflammatory processes. It is believed to play an important role in innate immunity ***(Pepys and Hirschfield, 2003).***

Aim of the work: This work was done to study the effect of dietary supplement of rosemary leaves as a strategy for amelioration of the side-effects of azathioprine.

Material and Methods:

The present study was carried out on thirty-two adult male albino rats, weighing 140-150 gm. They were housed in clean properly ventilated cages under the same environmental condition, with free access to food and water throughout the period of the experiment which was ten days. The animals were divided equally into four groups:

Group I: Control group.

Group II: Rosemary group. Animals received a daily oral dose of Rosemary leaves extracts for 10 days. Ten grams of rosemary leaves were added to 100 ml of boiled water, the extract was filtered and given to animals at a volume of 10 ml/kg BW/day through an osphageo-gastric tube ***(Amin and Hamza, 2005).***

Group III: Azathioprine group. Animals received a single dose of AZA (50mg/kg BW) intraperitoneally 24 hours before the end of the experiment ***(Amin and Hamza, 2005).***

GroupIV: Rosemary azathioprine group. Animals received oral doses of rosemary leaves aqueous extracts and azathioprine as in the previously mentioned regimen. At the end of the experiment all animals were fasted for 12 hours, anesthetised by ether, and blood samples were collected from retro-orbital sinus for estimation of:

- Serum Urea according to ***Fawcett and Scott, (1960)***.
- Serum creatinine according to ***Schimeister et al., (1964)***.
- Serum C-reactive protein according to ***Rifai et al., (1999)***.
- Serum amylase activity according to ***Mifflin et al., (1985).***
- Serum TNF-α level by ELISA according to ***Englmann et al., (1990).***

Statistical Analysis: All statistical analysis was computed by SPSS version 14.

The values obtained were revealed as mean ± S.E. Data were analyzed using student's‘t’-test and results were considered significant at $P < 0.05$.

Results

Regarding the kidney function; Serum urea was significantly deceased in rosemary group, significantly increased in AZA group while it

was insignificantly changed in rosemary AZA group compared to the control group. Serum creatinine level was significantly decreased in all groups compared to the control group. (Table 1, Fig 1, 2).

C-reactive protein was significantly increased in AZA group, insignificantly changed in rosemary group and insignificantly increased in rosemary AZA group when compared to the control group (Table 1, Fig 3).

The serum amylase and TNF-α were significantly increased in azathioprine group, while their levels were insignificantly changed in rosemary group and in rosemary AZA group compared to control group (Table 1, Fig 4, 5).

Comparing rosemary AZA group with AZA group, the levels of serum urea, CRP, amylase and TNF-α were significantly decreased while serum creatinine was insignificantly changed. On the other hand, all parameters were insignificantly changed in rosemary AZA group compared to rosemary group (Table 2).

Table (1): Mean±SE of measured parameters in studied groups compared to control.

Group / Parameter	Control	Rosmary		Azathioprin		Rosemary Azathio	
	mean±S.E	mean±S.E	P value	mean ±S.E	P value	mean±S.E	P value
Urea (mg/dl)	49.72±5.33	45.80±4.40	P>0.05†	117.38±23.62	P<0.05*	62.66±11.1	P>0.05†
Creatinine (mg/dl)	2.42±0.43	1.41±0.06	P<0.05*	0.63±0.13	P<0.05*	0.96±0.22	P<0.05*
CRP (mg/L)	0.24±0.03	0.23±0.02	P>0.05†	0.29±0.01	P<0.05*	0.25±0.02	P>0.05†
Amylase (U/ml)	256.12±60.1	217.38±23.6	P<0.05*	650.12±43.31	P<0.05*	216.4±50.2	P>0.05†
TNF-α (pg/ml)	1.07±0.26	0.52±0.1	P>0.05†	1.93±0.08	P<0.05*	0.84±0.1	P>0.05†

* significant †insignificant

Table (2): Statistical comparison between rosemary AZA group, rosemary group and azathioprine group.

Groups	Rosemary AZA versus Rosemary		Rose AZA versus Azathioprin	
Parametes	T-test	P value	T-test	P value
Urea (mg/dl)	1.359	P>0.05†	2.098	P<0.05* decreased
Creatinine (mg/dl)	0.623	P>0.05†	0.041	P>0.05†
CRP (mg/L)	0.707	P>0.05†	1.789	P<0.05* decreased
Amylase (U/ml)	1.21	P>0.05†	5.803	P<0.05* decreased
TNF-α (pg/ml)	1.43	P>0.05†	5.060	P<0.05* decreased

* significant †insignificant

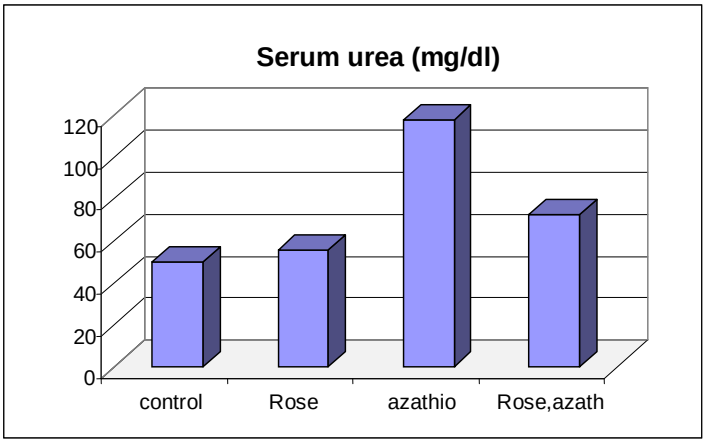

Fig (1): Serum urea (mg/dl) in all studied groups.

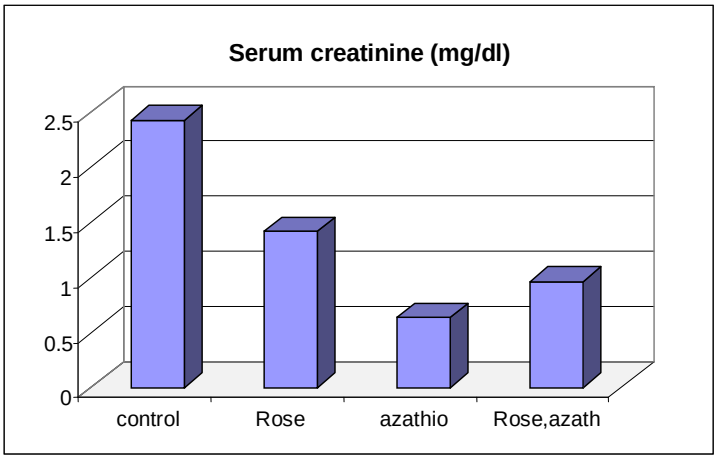

Fig (2): Serum creatinine (mg/dl) in all studied groups.

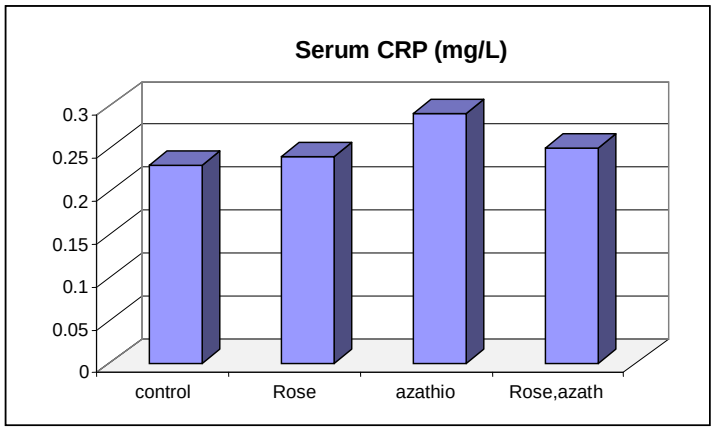

Fig (3): Serum CRP (mg/L) in the all studied groups.

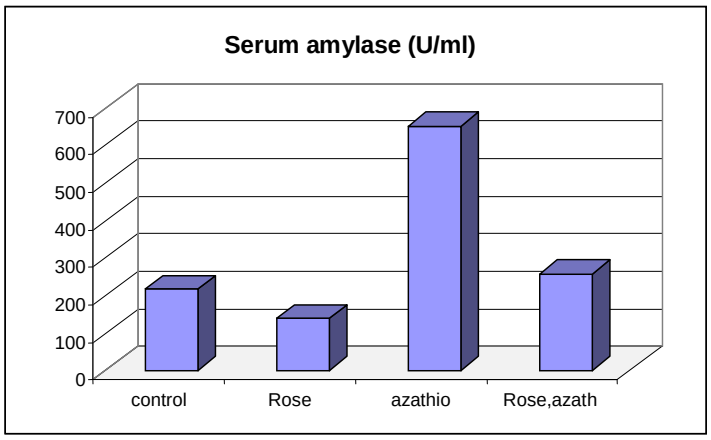

Fig (4): Serum amylase (U/ml) in all studied groups.

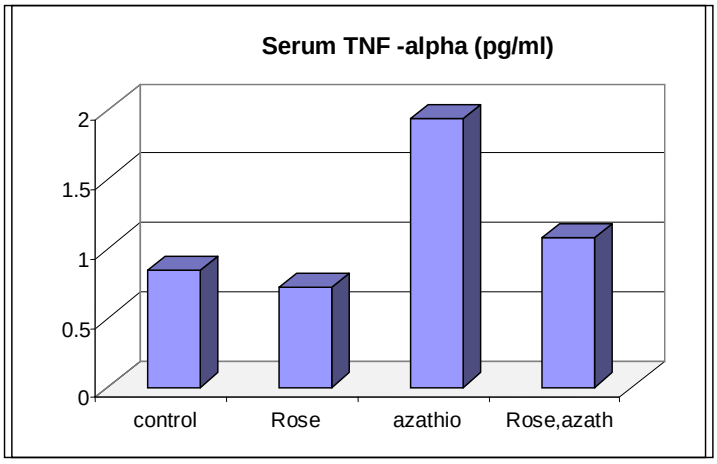

Fig (5): Serum TNF- α (pg/ml) in all studied groups.

Discussion:

Azathioprine, is widely used to treat malignancies, rheumatic diseases, dermatologic conditions, inflammatory bowel disease, and solid organ transplant rejection. However, thioprine drugs have a relatively narrow therapeutic index and are capable of causing life-threatening toxicity, most often myelosuppression ***(Sahasranaman et al., 2008).***

Although azathioprine is an immunosuppressant used in the treatment of several diseases, it has been reported to be hepatotoxic. Intra-peritoneal injection of AZA resulted in not only lymphocyte suppression but also toxicity to bone marrow, gastrointestinal tract, and liver ***(Amin and Hamza, 2005).***

Rosemary extracts are widely used in the food. Their major bioactive components have shown antioxidant, antimicrobial, anti-inflammatory, antitumorigenic and chemopreventive activities ***(Bozin et al., 2007)***.

In this study, it was noticed that azathioprine group showed a statistically significant increase ($P< 0.05$) of TNF-α, amylase enzyme, C-reactive protein and serum urea; while serum creatinine was significantly decreased ($P< 0.05$) compared to control group.

The increased level of blood urea does not indicate the presence of renal toxicity since serum creatinine did not increase. Blood urea elevation may be attributed to endogenous protein breakdown due to a possible increased tissue wasting ***(El-Beshbishy et al., 2010).***

The results of this study was in agreement with those of ***Bozin et al. (2007)*** who explained the occurrence of pancreatic inflammation after the daily doses of azathioprine as proved by the decreased macroscopic score. ***Alexander and Dowling (2005)*** reported that the traditional immunosuppressive agent, azathioprine, had been reported to induce pancreatitis which is manifested by elevation of serum amylase. Moreover, ***Trivedi and Pitchumoni (2005)*** classified azathioprine as a class I drug that induces pancreatitis.

On the contrary, ***Salmaggi et al. (1997)*** found that azathioprine has a strong anti-inflammatory effect and has also been shown to inhibit TNF-α.

The rosemary AZA group showed an insignificant change in all parameters except serum creatinine showed significant decrease ($P<0.05$) compared to those of control.

Comparing rosemary AZA group with AZA group levels of all measured parameters were significantly decreased ($P<0.05$) except serum creatinine was insignificantly increased.

In agreement with these results, ***Eknoyan et al. (2003)*** reported that rosmaric acid had been shown to decrease blood urea nitrogen and serum creatinine. ***Hoppel (2003)*** explained that the rosemary aqueous extract increased urinary excretion of urea nitrogen and creatinine, may be probably due to increased glomerular filtration.

Ghazalah and Ali (2008) suggested that rosemary may cause amendatory effect on renal and hepatic functions. They found that creatinine level was reduced by dietary rosemary leaves compared to controls.

Carnosic acid of rosemary markedly suppressed TNF-α, as well as the expression of inducible nitric oxide synthase and cyclooxygenase-2, phosphorylated inhibitor-kappaB, and nuclear factor-kappaB /p65 in a dose-dependent manner ***(Kuo et al., 2010).***

Bustanji et al. (2010) found that all the rosemary compounds (rosmarinic acid, chlorogenic acid, caffeic acid and gallic acid) were able to inhibit the pancreatic lipase activity in a dose dependent manner, but with different potencies.

Rosemary extracts and their triterpenes (ursolic acid, oleanolic acid and micromeric acid) have been shown to exert anti-inflammatory activity in vivo ***(Altinier et al., 2007)***.

Therapeutic concentrations of rosemary extracts significantly inhibited cytokine production in lymphocytes (TNF-α, IL-1β, IL-4 and IL-5) and in monocytes (TNF -α, IL-1β, IL-6 and IL-8) with less effects on chemotactic cytokines ***(Juergens et al., 2004).***

Cheung and Tai (2007) found that crude ethanolic rosemary extract has differential anti-proliferative effects on human leukemia and breast carcinoma cells. They found that ethanolic rosemary extract of 1/1000 and 1/500 dilutions did not affect TNF-α, IL-1beta, iNOS and COX-2 mRNA expression.

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دور مستخرج أوراق الروزمارى كعامل وقائى ضد السمية الناتجة من الأزاثيوبرين فى الجرذان

هالة محمد طاهر الموجى* ,جيهان أحمد يوسف**
أقسام الكيمياء الحيوية الطبية* والفسيولوجى**,كلية الطب (بنات)جامعة الأزهر

يتواجد نبات الروزمارى بكثرة على طول سواحل البحر المتوسط . وأوراق الروزمارى ومستخرجها لها دور مؤثر كمضاد للأكسدة ومانعة للإلتهابات,كما أنها تستخدم كمضاد للتقلصات , مسكن للألم , مضاد للروماتيزم و طارد للبلغم. ويرجع نشاط الروزمارى إلى محتواه من الزيوت الضرورية. ويعمل دواء الأزاثيوبرين على إخماد فعالية جهاز المناعة, ويستخدم فى كثير من الأمراض , ومن أهم الأعراض الجانبية الناتجة عن إستخدامه إلتهاب البنكرياس.

كان الهدف من إجراء هذا البحث هو دراسة تأثير تناول مستخرج أوراق الروزمارى فى تحسين الأعراض الجانبية للأزاثيوبرين.

وتم إجراء البحث على 32 من الجرذان الذكور تم تقسيمها بالتساوى إلى أربعة مجموعات:

المجموعة الاولى : المجموعة الضابطة

المجموعةالثانية : التى تناولت جرعة يومياً من مستخرج أوراق الروزمارى .

المجموعةالثالثه: التى تم حقنها بجرعة واحدة من الأزاثيوبرين .

المجموعةالرابعه: التى تناولت جرعة يومياً من مستخرج أوراق الروزمارى لمدة تسعة أيام ثم تم حقنها بجرعة واحدة من الأزاثيوبرين .

وقد تم إجراء البحث على مدارعشرة أيام و بعدها تم أخذ عينات دم من جميع الجرذان بعد تخديرها ثم تم قياس مستوى كل من اليوريا, الكرياتينين, البروتين التفاعلى-ج, إنزيم الأميليز و عامل الأورام التحللى-ألفا فى مصل هذه العينات. وقد أظهرت النتائج قدرة مستخرج أوراق الروزمارى على تحسين الأعراض الجانبية للأزاثيوبرين, حيث وجد نقص فى مستوى كل من الكرياتينين, إنزيم الأميليز و عامل الأورام التحللى-ألفا فى المجموعة الرابعة التى تناولت جرعة يومياً من مستخرج أوراق الروزمارى قبل حقنها بالأزاثيوبرين.

ومن هنا نستخلص أن مستخرج أوراق الروزمارى له القدرة على تحسين الأعراض الجانبية للأزاثيوبرين, ولذلك ينصح بتناوله عند العلاج بالأزاثيوبرين.