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INVESTIGATION ON THE COUNTERACTING EFFECT OF SPIRULINA AGAINST POTENTATED SULFONAMIDE'S (COTRIM DS®) SIDE EFFECTS IN RAT

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ABSTRACT

An experiment was conducted to investigate the counteracting effects of spirulina in Long Evans rats exposed to oral potentiated sulfonamide administration. 20 rats were randomly assigned into four equal groups (A, B, C and D) and were fed with standard broiler pellet (25g/rat/day) throughout the experimental period of 60 days. Rats of Group A were fed only with pellet without any experimental diet and were defined as control. Rats of Group B were treated with potentiated sulfonamide @ 96 mg/rat/day orally whereas Group C was treated with potentiated sulfonamide @ 96 mg/rat/day plus spirulina (*Spirulina maxima*) @ 50 mg/rat/day orally (low dose spirulina). In Group D, potentiated sulfonamide and spirulina (*Spirulina maxima*) were given through feed @ 96 mg/rat/day and @ 100 mg/rat/day (high dose spirulina) respectively. Hematological parameters (TEC, Hb and absolute count of lymphocyte, neutrophil and eosinophil) and hispathological profile of liver and kidney were recorded. The investigation revealed that the oral administration of sulfonamide significantly ($p<0.01$) decreased the TEC (5.93±0.24) value, number of lymphocyte (581.76±3.70) and neutrophil (581.76±3.70) compared to other treated groups and control group. On the other hand significant ($p<0.01$) increase (422.86±2.34) in eosinophil population has been found in rats fed on sulfonamide irrespective of spirulina supplementation on the final day of experiment compared to other treated group and control group. From this experiment it is evidenced that spirulina has a potential counteracting effect against sulfonamide. Histopathology of kidney and liver was done at the end of experiment (60 days) and no significant change was found except in the kidney of Group B and C.

Key words: Rat, sulfonamide, spirulina, hematological parameters and histopathology.

INTRODUCTION

Sulfonamide is a widely used antibiotic. They work by inhibiting normal bacterial utilization of para-aminobenzoic acid (PABA) for the synthesis of folic acid, an important metabolite in DNA synthesis. Bacterial resistance to sulfonamides is caused by mutation of folic acid enzyme that inhibits PABA from binding and block folic acid synthesis (Prescott and Baggot, 1993). Sulfonamides have several side effects. These have adverse reactions in elderly patients including severe skin reactions, generalized bone marrow suppression and decreased platelets (Agarwal, 1992). Moreover, these have gastrointestinal side effects (Bulgin *et al.*, 1991), hypersensitivity side effects (Noli *et al.*, 1995), hematologic side effects (Weiss and Adams, 1987), renal side effects (Duffee *et al.*, 1984) and hepatic side effects (Twedt *et al.*, 1997).

Spirulina (*Asthrospira platensis)* is a blue green algae (Ciferri and Tiboni, 1985). It is an ideal anti-aging food; concentrated with nutrient value, easily digestible and loaded with antioxidant. It is rich with beta carotene food, a full spectrum of ten mixed carotinoids (Dillon *et al.,* 1995). It has also many other beneficial effects to the body for e.g. it is a rich source of iron, magnesium and other trace minerals (Johnson and Shubert, 1986). It also improves immune function (Pugh *et al.,* 2001), increases absorption of nutrients from foods and helps to protect against infection (Ozdemir *et al.,* 2004). Spirulina reduces kidney toxicity caused by the heavy metal mercury and several pharmaceutical drugs (Mohan *et al.,* 2006) also helps to fight against cancer (Schwartz *et al.,* 1988) by promoting the release of tumor necrosis factor alpha, a chemical in the body that attacks tumor cells. It appears to increase production of anti-inflammatory chemicals known as interferons and interleukins (Lee and Werth, 2004). Consequently, it decreases or prevents some allergic responses and blocks the release of histamine from mast cells during an allergic reaction (Mao *et al.,* 2005). By blocking histamine release, it prevents or lessens histamine's effects, which include blood vessel expansion, muscle contraction, and stomach acid production. It is the highest source of vitamin B-12, vitamin B Complex, essential for healthy nerves and tissue especially for vegetarians (Maranesi *et al.,* 1984).

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Spirulina was found to reduce the inflammation involved in arthritis in geriatric patients by stimulating the secretion of interleukin-2, which helps in regulating the inflammatory response (Park *et al.*, 2008). In addition, several studies have shown that spirulina produces several biological effects such as antitumor (Mittal *et al.*, 1999), antimicrobial (Hayashi *et al.,* 2007), mettaloprotective (Sharma *et al.,* 2007) effects. In Bangladesh there is no available research data of sulfonamides and spirulina on laboratory animals.

The present hematological study is a preliminary work in rats to provide guidance in carrying out further detail study on sulfonamides in Bangladesh. So this pioneering works has been taken with a view to study the effects of sulfonamide on some selected blood parameters (TEC, Hb content and absolute count of lymphocyte, neutrophil and eosinophil) and histology of kidney and liver. This study was undertaken also to oversee any counteracting tendency of spirulina against the sulfonamide action in rats.

MATERIALS AND METHODS

Animals

Two months old 20 Long Evans male rats with a mean initial body weight of 30±2g were purchased from Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh and were maintained well with feed and water during the experimental period. Before being used in the experiment, rats were adapted for 3 days in order for them to get used to the environment. All rats were maintained by following the “Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985).

Experimental protocol and diets

All the rats were randomly assigned into four equal groups (A, B, C and D) and were fed with standard broiler pellet (25g/rat/day) throughout the experimental period of 60 days. Rats of group A were fed only with pellet without any supplementation and were defined as control. Rats of group B were treated with sulfonamide @ 96 mg/rat/day orally with water whereas group C was treated with potentiated sulfonamide @ 96 mg/rat/day plus spirulina (*Spirulina maxima*) powder @ 50 mg/rat/day (low dose spirulina) with water. In group D, sulfonamide and spirulina powder were given with water @ 96 mg/rat/day and @ 100 mg/rat/day (high dose spirulina), respectively.

Hematological parameters

The blood was collected from tail for the measurement of blood parameters on the first day of the experiment. A set of sterile test tubes containing anticoagulant (3.8% Sodium Citrate Solution) at a ratio of 1:10 were used in blood collection. At the end of the experimental period (60 days), the rats were euthanized by carbon dioxide asphyxiation and blood was withdrawn by cardiac puncture using a 22 gauge needle and a 5 mL syringe. The blood was kept at 4°C till examination was done. Hematological studies were performed in Physiology laboratory within two hours of blood collection. Blood parameters were studied as per method described by Lamberg and Rothstein (1977).

Histopathology

Histopathology was performed in the Anatomy and Histology laboratory. The whole liver and kidney were excised from each animal, immersed in chilled phosphate-buffered saline and blotted dry. A 4 mm^3 section of the liver and kidney were placed into a histological cassette. The cassette containing the liver and kidney section of each animal was individually immersed in a 10% (v/v) buffered formalin phosphate solution for fixing and subsequent staining. The formalin-fixed, paraffin-embedded tissue samples were ultra sectioned (4–5 µm thickness), stained with Verhoeff’s stain and examined under a light microscope (Luna, 1968).

Statistical analysis

All data were expressed as mean ± SD of 5 rats of each group. The data on hematology was subjected to statistical analysis by applying Completely Randomized Design (CRD).Statistical significance was set at $p < 0.05$ (Mostafa, 1989).

RESULTS AND DISCUSSION

Hematological parameters

The values of Total Erythrocyte Count (TEC), Hb content, absolute count of lymphocyte and eosinophil are presented in Table 1. Apparently the TEC values and Hb content have been found reduced among all the sulfonamide treated groups, the significant ($p<0.01$) reduction has been recorded in the rats fed on normal ration supplemented by sulfonamide only compared to other treated group. This is an indication of negative impact of this drug on erythrocytic parameters. This finding is in close agreement with others (Kaneene and Miller, 1992; Velanker *et al.,* 1999; Plaford *et al.,* 2001). At the same time spirulina in the diet is thought to invigorate the liver function that indirectly enhances erythropoiesis (Guyton and Hall, 2006). The greatly decrease in TEC values and Hb content in sulfonamide treated rats compared to sulfonamide and spirulina treated and control group may be an indication of suppressive action of this drug on bone marrow.

The less reduction on TEC values and Hb content in other two groups might be the consequence of compensatory reaction of spirulina against sulfonamide since spirulina is shown to have some important erythropoietic factors like vitamins and minerals (Johnson and Shubert, 1986; Maranesi *et al.* 1984).

Table 1. Effect of oral administration of sulfonamide and spirulina on blood parameters

Para-meters	**Groups**							
	A (Control Group)		B (Sulfonamide @ 96 mg/rat/day)		C (Sulfonamide @ 96 mg/rat/day and spirulina @ 50 mg/rat/day)		D (Sulfonamide @ 96 mg/rat/day and spirulina @ 100 mg/rat/day)	
	Day '0'	Day '60'	Day '0'	Day '60'	Day '0'	Day '60'	Day '0'	Day '60'
TEC (million/mm^3)	7.5 ±0.2	7.6± 0.2	7.6± 0.2	5.9± 0.2**	7.5±0.2	6.573± 0.29	6.6±0.3	7.3±0.3
Hb (mg/dl)	12.5 ±0.1	12.5± 0.2	12.5± 0.2	10.7± 0.2**	12.6±0.1	11.433± 0.321*	12.5±0.3	12.3± 0.1
Lymphocyte/mm^3 (absolute count)	6483.8 ±8.9	6510.2± 6.7	6477.4± 8.4	5943.9 ±11.9**	6474.8± 7.8	6141.7± 15.9*	6478.5±7.4	6360.7± 15.1*
Neutrophil/mm^3 (absolute count)	1108.2±2.4	1112.1± 5.8	1109.53± 12.5	581.76± 3.7**	1108.5± 11.4	754.4± 12.2**	1110.5±7.3	1032.9± 2.7**
Eosinophil/mm^3 (absolute count)	294.9 ±1.7	298.1± 2.6	295.2± 2.6	422.9± 2.3**	294.8±2.6	402.7± 4.2**	295.7±2.5	361.9± 5.3**

Values given above represent the mean ± standard deviation (SD) of five rats (n=5)
* = Significant at $p<0.05$ ** = Significant at $p<0.01$

All the treated groups have shown decreased lymphocyte population. Among these three groups, the lymphocyte number in rats fed on sulfonamide plus spirulina has been little higher than in rats treated with only sulfonamide. These findings correspond to other researchers (Brenner *et al.,* 1995) who found that sulfonamide administration causes leucopenia in animal. This gives an impression that spirulina has an invigorating effect on lymphoid organs.

The neutrophilic population has been seriously decreased in all sulfonamide treated groups irrespective of spirulina supplementation. Similar findings are observed by others (Levi *et al.,* 1998) who found that sulfonamides administration in lactating cow causes leucopenia. This is an indication of irreversible damage to the bone marrow cells committed for neutrophil which is beyond the regenerative effect of spirulina on the bone marrow.

The eosinophil number has been found greatly higher in all sulfonamide treated rats no matter how much spirulina they received. The significant increase of eosinophil population in all treated groups might be cause of hypersensitivity reaction exerted by sulfonamide that is beyond to counteracting capacity of spirulina.

HISTOPATHOLOGY

In histopathology, no specific lesions were found in the liver of all treated groups (Figure 1) as compared with the control group. However, a slight degenerative change in the renal parenchyma in Group B (Figure 2) and hypercelluarity in kidney glomerulus in Group C (Figure 2) were observed.

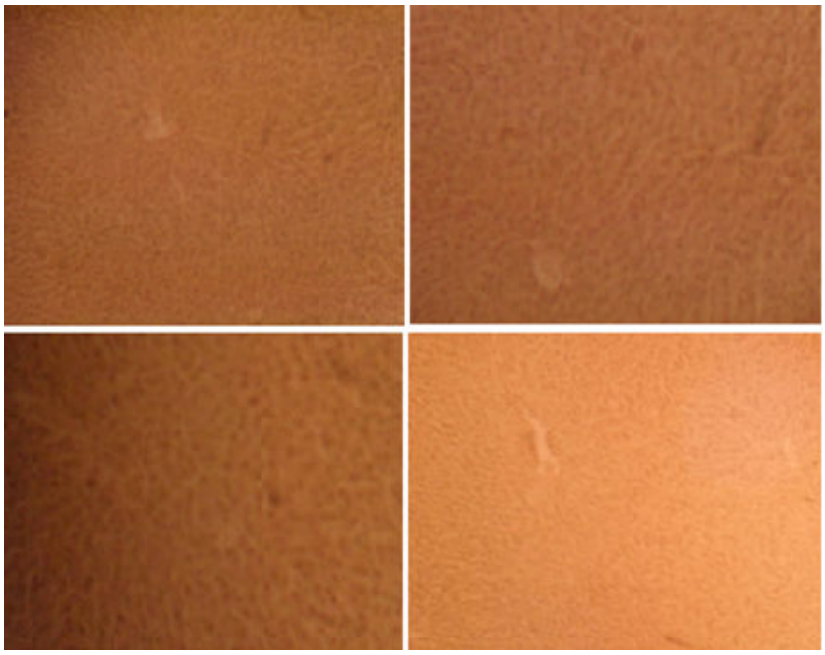

Figure 1. Histopathology of liver of rat (Verhoeff's Stain). Photographs were taken under microscope (Olympus) at 100X magnification. Liver showing normal texture in all groups of rat.

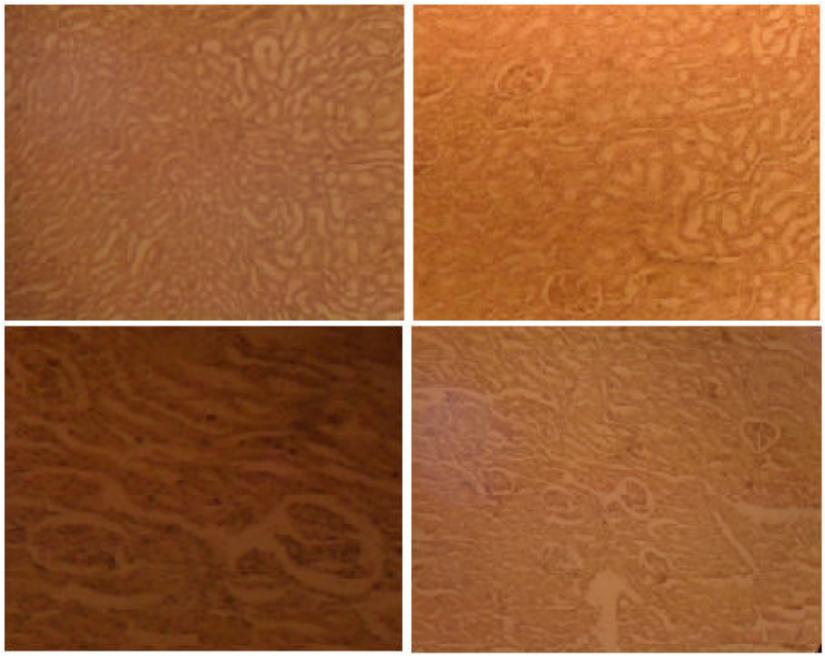

Figure 2. Histopathology of kidney of rat (Verhoeff's Stain). Photographs were taken under microscope (Olympus) at 100X magnification. Kidney of Group A (control) showing normal texture, slight degenerative changes in kidney of Group B, Hypercellularity of glomerulous in kidney of Group C and no remarkable change in kidney of Group D.

From the above findings we may conclude that administration of sulfonamide has several adverse effects in rats and spirulina has beneficial effect on hematopoiesis.

Of course, the present study is a preliminary work in laboratory animal in Bangladesh. In order to have a clear idea about sulfonamide on hematopoiesis the present work should be further extended to detailed observation of bone marrow by radiographic intervention as well as painstaking histology.

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