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ORIGINAL ARTICLE

Effect of Spirulina (*Arthrospra platensis*) on blood uric acid, Hemoglobin and kidney histo-texture of mice treated with acetaminophen

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Abstract

Background: Acetaminophen is a medication used to treat pain and fever. It is typically used for mild to moderate pain relief. Spirulina is a biomass of cyanobacteria that cultivated worldwide and used as a dietary supplement or whole food. It is also used as a feed supplement in the aquaculture, aquarium, and poultry industries. The present study was undertaken to determine the effect of *Spirulina* on blood and kidney in mice.

Methods: Twenty Swiss Albino mice *(Mus musculus)* were randomly divided into 4 equal groups' viz., A, B, C, and D. Group A was kept as control and fed only normal diet. Group B was supplemented with acetaminophen where as group C was supplemented with acetaminophen and *Spirulina*, and group D was treated with *Spirulina*.

Results: The application of Acetaminophen did not have significant effect on hemoglobin and uric acid content. But the addition of *Spirulina* to the diet increased significantly ($P<0.05$) hemoglobin and uric acid level. Histopathological examination revealed that no significant changes were found in the kidney of all of treated groups in comparison with the mice of control group.

Conclusions: The research work suggests that long-term ingestion of acetaminophen does not exert a significant change in hemoglobin or uric acid level but spirulina supplemented diet may be associated with the rise in hemoglobin and uric acid level without affecting renal tissue texture in a significant manner.

Key words: *Spirulina*, Acetaminophen, mice, hemoglobin, uric acid, kidney

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Introduction

Acetaminophen or paracetamol (*N*-acetyl-para-aminophenol [APAP]) used as an analgesic and antipyretic agent. It is believed that selective inhibition of the enzyme COX-3 in the brain and spinal cord explains the effectiveness of paracetamol in relieving pain and reducing fever without having unwanted gastrointestinal side effects. It is a safe drug when given in therapeutic doses but its overdose is fairly common since it has narrow therapeutic index (Ramsay *et al.,* 1989). Acetaminophen nephrotoxicity is characterized by glutathione depletion and cellular necrosis (Richie *et al.,* 1992 and Tarloff *et al.* 1990) and increased toxicity is related to obesity. (Corcoran and Wong, 1987). *Spirulina* is a dietary supplement available in tablet, flakes and powder forms made from two species of cyanobacteria: *Arthrospra platensis* and *Arthrospra maxima*. It is an ideal antiaging food having concentrated nutrient value, easily digested and loaded with antioxidants and mixed carotenoids (Dillon *et al*., 1995). *Spirulina* improves immune function (Pugh *et al.,* 2001) and increase the production of 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). 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). *Spirulina* accelerates detoxification during fasting and cleansing. It significantly reduced kidney toxicity caused by the heavy metal mercury and several pharmaceutical drugs (Mohan *et al.,* 2006). *Spirulina* help to fight cancer by promoting the release of tumor necrosis factor alpha, a chemical in the body that attacks tumor cells (Schwartz *et al.,* 1988). It actually enhances the body's ability to generate new blood cells and increases disease resistance in animals, even in very small doses. So it can be used as an alternative therapy to help replace overused antibiotic drugs in animal feeds. The study has been conducted to know the effect of acetaminophen on blood and kidney histo-texture and ameliorating effect of Spirulina against acetaminophen induced changes.

Materials and Methods

Experimental Animals

The experiment was conducted in the Department of Physiology, Bangladesh Agricultural University (BAU), Mymensingh. The mice were purchased from Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh. Before being used in the experiment, mice were adapted for 7 days in order for them to get used to the environment. All mice were housed in a compartmented rectangular metallic cage (9× 11 ×7 cubic inches) wrapped with wire mesh. The cages were kept in well ventilated room at 28 $\pm 2^0$C and a relative humidity of 70-80% with natural daylight.

Experimental drug

A modern drug was purchased from local market and this was: Tablet Napa® from Beximco pharma. (Each tablet contains acetaminophen (Paracetamol) -500 mg).

Experimental diet

The feed was supplied with Spirulina (*Arthrospra platensis*) which was collected from Department of Pharmocology, Bangladesh Agricultural University (BAU), Mymensingh.

Experimental designs

A total twenty Swiss Albino mice *(Mus musculus)* of 2 months age with an average body weight of 20-25 g were used. At first, the mice were randomly divided into 4 equal groups viz. A, B, C, D. Mice of all groups were supplied with standard mouse pellet viz.(3 g/ mice /day) and fresh drinking water *ad libitum* throughout the experimental period of 90 days. Group A was kept as control and fed with normal mouse pellet. Group B was treated with acetaminophen at a dose rate of 900 mg /kg /body wt, where as group C was fed with Acetaminophen at a dose rate of 900 mg /kg /body wt and *Spirulina* at a dose rate of 800 mg /kg /body wt respectively. Group D was fed with *Spirulina* at a dose rate of 800 mg /kg body wt.

Management Practices

The feed was supplied daily to the mice and fresh drinking water was made available. In order to prevent spoilage, feeds were kept in air tight poly packed sac. Mice cage were cleaned regularly and proper hygienic and sanitary measures were adopted during the experimental period.

Collection of Blood

On the 1st day (after acclimation) and on the 90th day of experiment, blood samples were collected by sacrificing the mice. Before the collection of blood samples, the mice were anesthetized by placing them into a desiccator containing cotton soaked with chloroform and the blood was collected by a syringe directly from the abdominal aorta. The blood was then taken in the test tube containing anticoagulant and hematological tests were performed immediately and the remaining amount of blood was used for the collection of serum. Whole blood sample was used for estimation of hemoglobin (Hb) content and the serum was used for the measurement of Uric acid.

Hb (Hemoglobin) estimation and measurement of blood analytes

The Hb concentration was determined according to the procedure described by Ghai (1999).

The uric acid was measured by the auto analyzer apparatus (Reflotron uric acid, Roche, Germany) using commercially available Reflotron kits (Roche Diagnostics, Germany).

Histopathological study

On the 90th day of experiment, kidney samples from each group of mice were collected after complete removal of blood by perfusion with phosphate buffered saline and kept in 10% neutral buffered formalin for 15 days. The well fixed tissue sections were processed, paraffin-embedded, sectioned and were routinely stained with Hematoxylin and Eosin (H&E) stain as per standard procedure (Robert *et al.*, 2014). Photographs of the sections were taken at different magnifications in a Nikon Eclipse E600 Binocular Microscope, fitted with Nikon Digital Camera model DXM1200F, Japan.

Statistical analysis

All data were subjected to statistical analysis using SPSS program by one-way ANOVA followed by post-hoc Turkey's test.

Results and Discussion

Hemoglobin

The mice in group B (supplemented with Acetaminophen) and group C (supplemented with Acetaminophen + *Spirulina*) showed no significant change in the hemoglobin content during pre-treatment and post-treatment compared to that in control group. But the amplitude of increase in hemoglobin content was found significantly higher ($P<0.01$) in group D (supplemented with *Spirulina*) compared to the value observed in control group. The result revealed that, the application of Acetaminophen did not have significant effect on hemoglobin content in mice when application was made for 90 days. But the use of *Spirulina* in the diet induced a significant increase in hemoglobin. From this result it can be concluded that 90 days application of acetaminophen at the applied dose is not dangerous enough to induce any alteration on hemoglobin mass. At the same time *Spirulina* in the diet is thought to invigorate the liver function that indirectly enhances erythropoiesis (Guyton and Hall, 2006). This finding is in almost close agreement with the work reported by Ismail *et al.* 2009.

Table 1. Effect of Acetaminophen and *Spirulina* on hemoglobin (gm %) in mice

		Hemoglobin (gm %)	
Groups	Treatment	Pre-Treatment	Post-Treatment
A	Control	8.60±0.05	8.85±0.23
B	Acetaminophen	8.11±0.20	8.49±0.05
C	Acetaminophen+*Spirulina*	8.22±0.18	8.70±0.20
D	*Spirulina*	8.72±0.12	8.81±0.26*

Values given above are Mean±SE of 5 mice

* Significantly increased ($P<0.01$)

Uric Acid

The post treatment level of uric acid in group B (supplemented with Acetaminophen) and group C (supplemented with Acetaminophen + *Spirulina*) were found significantly ($p<0.05$) higher than pretreatment values in all the mice that received Acetaminophen, Acetaminophen and *Spirulina*. The higher level of uric acid in these mice might be a case of nephrotoxicity by acetaminopohen where the excretory rate of uric acid has been reduced by renal insufficiency. The result observed here is in agreement with the work reported by Tarloff *et al.*, 1990. The post-treatment level of uric acid in group D (supplemented with *Spirulina*) was found non-significant than pre-treatment values in all the mice that received *Spirulina.*

Table 2. Effect of Acetaminophen and *Spirulina* on uric acid in mice

		Uric acid	
Groups	Treatment	Pre-Treatment	Post-Treatment
A	Control	0.61±0.03	0.68±0.03
B	Acetaminophem	0.60±0.18	0.71±0.02 *
C	Acetaminophem+*Spirulina*	0.62±0.02	0.72±0.01 *
D	*Spirulina*	0.61±0.03	0.71±0.02

Values given above are mean±SE of 5 mice

* Significantly increased ($P<0.05$)

Histopathological Observation

In the present study, the non-specific histopathological changes were found in the kidney of all treated group. In acetaminophen treated group, tubular necrosis, vacuoles and desquamating cells were found(shown in the picture using arrows). These findings are almost similar to the findings of Gulnaz *et., al* (2010) who found tubular necrosis, vacuolar degeneration of tubules, epithelial desquamation and intra-luminal casts after treatment with acetaminophen in rats. Abdel- zaher (2007) observed moderate cloudy swelling of proximal convoluted tubules and vacuolar degeneration of distal convoluted tubules after administration of acetaminophen. In group C and D, there were slight necrotic changes in tubule. However; these histopathological changes were not significant in the treated group as compared with control.

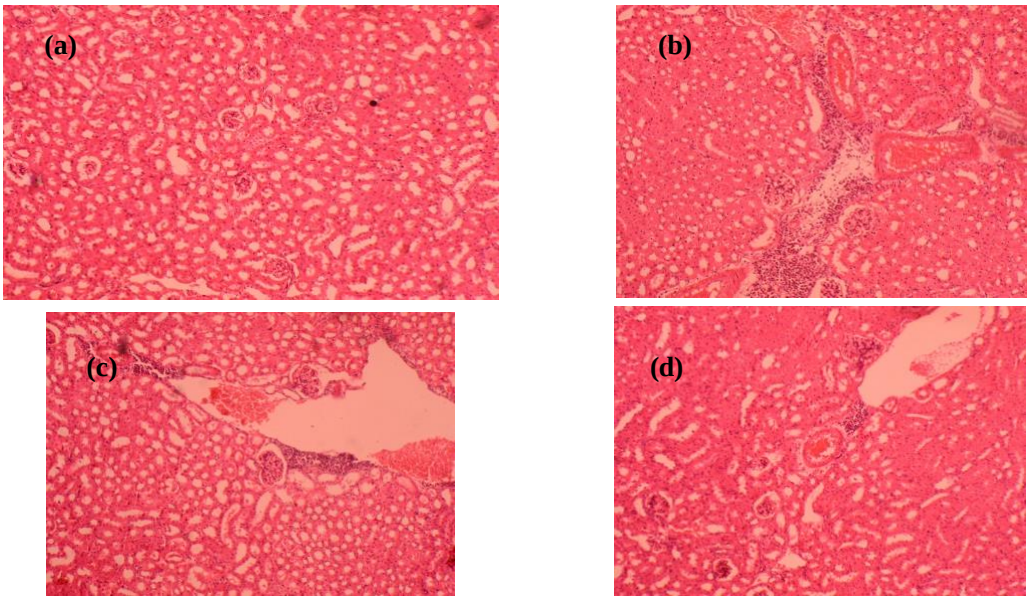

Fig 1. Histopathological section of kidney of mice (H&E x 100), (a) control group; (b) Acetaminophen treated group; (c) Acetaminophen & *Spirulina* treated group; (d) *Spirulina* treated group

Conclusions

The study reveals that prolonged ingestion of acetaminophen causes nephrotoxicity and histological changes in the kidney. *Spirulina* could be used as a dietary supplement with minimal biological hazard that subsided the unwanted changes in the kidney tissue by its toxin neutralizing and hemopoietic function. Further work is needed to isolate the active principle of *Spirulina* which is actually related to induce its impact on the body.

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