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Original article

Suppressive effect of *Spirulina fusiformis* on diclofenac-induced hepato-renal injury and gastrointestinal ulcer in Wistar albino rats: A biochemical and histological approach

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ABSTRACT

Context: The non-steroidal anti-inflammatory drug (NSAID), diclofenac causes hepato-renal toxicity and gastric ulcer. The aim of this study was to investigate the protective effect of *Spirulina fusiformis* on Diclofenac-induced toxicity in Wistar albino rats.
Methods: Rats were treated as follows: normal control (group I); diclofenac (50 mg/kg b.w., *i.p.*) treated rats (group II); diclofenac-induced (50 mg/kg b.w., *i.p.*) rats treated with *Spirulina fusiformis* (400 mg/kg b.w., *p.o.*) (group III); diclofenac-induced (50 mg/kg b.w., *i.p.*) rats treated with silymarin (25 mg/kg b.w., *p.o.*) (group IV); *Spirulina fusiformis* (400 mg/kg b.w., *p.o.*) alone treated rats (group V). Biochemical (liver and kidney functional markers) and antioxidant parameters (enzymic and non-enzymic antioxidants) were measured in the blood and tissue homogenates of the rats. Evaluation of intestinal ulcer score and assessment of liver and kidney histology were also done.
Discussion: Alterations in the levels of biochemical and antioxidant assays and histopathological changes in liver and kidney proved the toxic effect of diclofenac. The ulcer score was significantly increased in the diclofenac treated rats. *Spirulina fusiformis* showed to reduce such changes and was able to restore normal antioxidant status in the rats.
Conclusion: Our study proves the hepato-renal and gastroprotective activity of *Spirulina fusiformis* in diclofenac-treated rats.

1. Introduction

Liver has been found to be involved in several vital functions of the body. It is involved in detoxification of xenobiotics and excretion of endogenous and exogenous waste. It also plays an important role in the metabolism of carbohydrate, fats and protein, bile secretion and urea formation [1]. Healthy liver is responsible for the maintenance of human growth, fertility and good health. As Liver is the major site for biotransformation and detoxification, it receives many toxic metabolites [2–4]. The toxic substances generate reactive free radicals which cause lipid peroxidation by linking covalently with membrane lipids [5]. Membrane permeability is thus altered by lipid peroxidation thereby causing liver damage. Liver disease has become a major health disorder in recent years [4,6]. The inbuilt antioxidant systems like superoxide dismutase (SOD) and tissue glutathione (GSH) would prevent the tissues from free radical attack. Liver damage is evidenced when there is excess production of reactive oxygen species. Drug-induced liver damage could be prevented by strengthening the in-built protective mechanisms [7].

More than 1000 pharmaceutical drugs have the ability to cause liver injury [8]. Therapeutic drugs such as antibiotics and non-steroidal anti-inflammatory drugs (NSAIDS) are known to cause hepato-renal toxicity. Diclofenac (DFC) has been reported to cause drug-induced liver injury in rat [8,9]. Long-term use and over-dosage of DFC leads to hepatotoxic effects [9]. It is a non-steroidal anti-inflammatory drug, which is used for the treatment of rheumatic diseases and also used as analgesic and anti-inflammatory agent. DFC is known to cause gastric, liver and brain toxicity [10]. Similar to other NSAIDS, DFC also causes mild to severe hepatotoxicity. DFC causes the opening of mitochondrial permeability pore through the uncoupling of oxidative phosphorylation

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thereby causing cellular damage [11]. Herbal plants are being used as medicines and dietary supplements as they cause minimal side effects [12,13]. Current research investigations are aimed at increasing the safety and efficacy of herbal drugs in the management and treatment of various ailments [14].

Spirulina fusiformis (SPI) is a blue green algae; a Cyanobacterium used as food supplement in humans and animals. There is no evidence of toxic effect on the usage of Spirulina. Spirulina is a rich source of vitamins, trace minerals, antioxidants and carbohydrate [15]. Spirulina is composed of low caloric, low fat and cholesterol free protein rich source. It is known to possess antioxidant, hypolipidemic and anti-inflammatory activities. The long time use of Spirulina would help reduce body fats. It also helps in regulating blood circulation [16]. Spirulina has been proved to be effective in diabetes and cardiovascular disease, without causing any changes in physiological, biochemical and pathological alterations [15,16]. Previous studies have shown that Spirulina possesses significant anticancer activity [17].

The aim of present study is to investigate the hepatoprotective effect of Spirulina on DFC induced-toxicity in Wistar Albino rats.

2. Materials and methods

2.1. Chemicals and reagents

DFC was purchased from Unique Pharmaceutical Laboratories Pvt. Ltd., Mumbai, Maharashtra, India. SPI capsules were obtained from Acumen Pharmaceuticals Pvt. Ltd., Puducherry, India. Silymarin (SLY) was purchased from Micro labs Pvt. Ltd., Solan, Himachal Pradesh, India. Commercial diagnostic kits for aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total and direct bilirubin, total cholesterol, high density lipoprotein (HDL), total protein, albumin, urea, uric acid, creatinine and plasma glucose were obtained from Span Diagnostics Ltd., Surat, Gujarat, India.

2.2. Animals

Thirty female Wistar albino rats weighing about 180–200 g were obtained from the animal house, VIT University, Vellore, Tamilnadu, India. The Animals were maintained in a temperature and light controlled room and housed six per cage. The rats were acclimatized for a week before the commencement of the experiment. The animals were allowed free access to commercial pelleted rat feed obtained from Hindustan lever ltd (Mumbai, India) and water. The experimental procedure was approved by the institutional ethical committee, VIT University, Vellore, India (VIT/IAEC/11th/October 10th/No.26).

2.3. Experimental design

The rats were divided into 5 groups of six rats each and treated as follows for duration of five days:

Group I: Normal control rats

Group II: Toxic control rats treated with DFC (50 mg/kg b.w./day, i.p) alone on 3rd and 4th day.

Group III: SPI (400 mg/kg b.w./day) given orally for 5 consecutive days and DFC (50 mg/kg.b.w./kg, i.p) on 3rd and 4th day.

Group IV: SLY (50 mg/kg b.w./day) given orally for 5 consecutive days and DFC (50 mg/kg.b.w./day, i.p) on 3rd and 4th day.

Group V: SPI (400 mg/kg b.w./day) alone treated rats.

The animals were sacrificed after last dosage using ether anesthesia. Blood was collected from the trunk and liver and kidney were procured for histopathological examination. The blood samples were centrifuged at 2000 rpm for 10 min to obtain serum.

2.4. Weight assessment

The body weights of the animals were measured before commencement of the experiment and also on each day of the treatment until sacrifice. The weight of liver and kidney were analyzed after the sacrifice. Liver index was calculated for each animal using the following formula:

$$\text{Liver index} = (\text{Liver weight}/\text{body weight}) \times 100\%$$

2.5. Assessment of hepatoprotective activity

Serum of the experimental rats were used to determine the levels of AST, ALT, ALP, total bilirubin, direct bilirubin, total protein, albumin, total cholesterol, triglycerides and high density lipoprotein using commercial diagnostic kits purchased from AutoSpan Diagnostics Ltd., India. The assays were carried out according to the manufacturer's protocol.

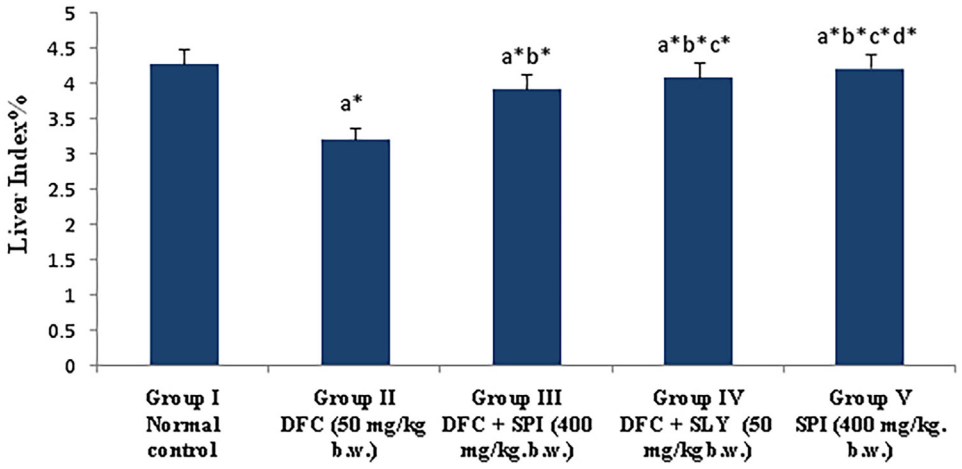

Fig. 1. Protective effect of SPI on liver index in DFC-treated rats.
Each value represents the mean ± SD of six rats. Comparisons were made as follows: a-group 1 vs groups 2, 3, 4, 5; b- group 2 vs group 3, 4, 5; c-group 3 vs group 4, 5; d-group 4 vs group 5. The symbols represent statistical significance at $^*p < 0.05$. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.