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International Journal of Current Research
Vol. 10, Issue, 04, pp.68430-68433, April, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

GASTROPROTECTIVE AND ANTIOXIDANT POTENTIAL OF GRIFFITHSIA PACIFICA KYLIN AGAINST INDOMETHACIN-INDUCED GASTRIC ULCER IN RATS

***Sulthana Begam, M.**

Research Scholar, Department of Zoology, Thiruvalluvar University, Serkadu, Vellore, Tamilnadu, India

ARTICLE INFO

Article History:
Received 23rd January, 2018
Received in revised form
17th February, 2018
Accepted 09th March, 2018
Published online 30th April, 2018

Key words:
Antioxidant; Gastric protective;
Griffithsia Pacifica Kylin;
Marine red algae; Indomethacin.

ABSTRACT

The aim of the present study is to evaluate the in vivo gastro protective and antioxidant activity of the marine red algae Griffithsia Pacifica Kylin (GPK) against indomethacin (IND)-induced gastric ulcer on experimental rats. The results revealed by the ethanolic extract of GPK produced significant reduction in gastric mucosal lesions, malondialdehyde (MDA), and tumour necrosis factor - α (TNF-α) associated with a remarkable increase in gastric juice, mucin content and gastric mucosal catalase (CAT), Nitric oxide (NO), and Prostaglandin E2 (PGE2) levels. The volume and acidity of the gastric juice decreased in pretreated rats. The GPK algae extract was elevated in the gastric juice of rats untreated has showed near normal levels in pretreated rats. The GPK were able to decrease acidity and increase the mucosal defense in the gastric area. Ranitidine (RAN) significantly increased pH value and decreased pepsin activity and gastric juice free and total acidity. The antiulcer effect was further confirmed histologically. Finally the current study justifying the traditional usage of these GPK to treat gastric ulcers.

Citation: Sulthana Begam, M. 2018. "Gastroprotective and antioxidant potential of griffithsia pacifica kylin against indomethacin-induced gastric ulcer in rats", *International Journal of Current Research*, 10, (04), 68430-68433.

INTRODUCTION

Gastric ulcer is most typical global disorders of the gastro intestinal tract, with increasing incidence and prevalence. It is a complex pluricausal disease and its etiology is still unclear. It is known to develop mainly due to the imbalance between the aggressive (acid-pepsin) and defensive factors (mucin secretion, cellular mucus, cell shedding and cell proliferation) (Sairam *et al*., 2003). Several pharmaceutical products have been employed for the treatment of gastro duodenal ulcer and peptic diseases, resulting in decreasing mortality and morbidity rates, but they are not completely effective and produce many adverse effects (Rates, 2001). In recent years an increasing number of compounds are isolated from marine algae that possess various biological activities (El Gamal, 2010; Guven and Percot, 2010; Li *et al*., 2008; Wijesekara *et al*., 2011). Griffithsia Pacifica Kylin is a well-known ceramaceous red algal genus, which has characteristic large vegetative cells visible to the unaided eye and thousands of nuclei in a single cell at maturity and has served as a useful tool for many developmental studies. Red Algae are orange to pink, 3-5cm tall, and thallus monosiphonus tuffed –filamentous, clearly visible to naked eye (Guiry *et al*., 2013). They are excellent source of biologically active phytochemicals, which include carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, sterols, tocopherol, and phycocyanins among others. Many of these compounds have been recognized to possess biological activity and hence beneficial for use in human and animal healthcare (Gamal *et al*., 2010). Some of the potential benefits include control of hyperlipidaemia, tumour, obesity and gastroprotective activity (Vishwamodia *et al*., 2009). In addition, it has been reported that ethanol extract of red sea weed exhibited the highest antioxidant activity (Yuan *et al*., 2005).The literature survey revealed that the Griffithsia Pacifica Kylin (GPK) could be the possible sources for obtaining potential algae products with gastroprotective and antioxidant properties. The present study is focused to examine the capacity of GPK algae extract for gastroprotective and antioxidant activity induced by indomethacin.

Corresponding author:* **Sulthana Begam, M.
Research Scholar, Department of Zoology, Thiruvalluvar University, Serkadu, Vellore, Tamilnadu, India.

Experimental

Preparation of Ethanol extract of GPK algae

Algal materials were collected from the Rameswaram, Tamilnadu, India and obtained fisher by catching method. The collected red algae were washed with tap water to remove salts and other adhering particles. The whole red algae was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol in soxhlet's

apparatus to 60'c. Cleaned GPK algae were shade dried and the completely dried material was weighed and grind coarsely in a mechanical grinder. In the present study we evaluate the biological potencies of marine red algae.

Drugs and chemicals

Indomethacin (IND), Tween 80, Ranitidine(RAN), Thiobarbi turic acid, 1,1,3,3-tetramethoxy-propane, trichlor oacetic acid, ethanol absolute and diethyl ether, Saline were the chemicals used in this study. Indomethacin and ranitidine was purchased from Micro labs, Tamilnadu, India., and the rest of the chemicals utilized were of analytical grade and were obtained from Ranbaxy Research Laboratory, Hyderabad, India.

Acute toxicity test

The albino Wistar rats were divided into six groups of six animals each and a group received saline (10 ml/kg) kept as normal control. A single dose of GPK extract was administered orally to group 2, 3 and 4 at doses of 50, 500, and 5000 mg/kg b.wt. respectively. The extract did not produce any toxic symptoms of mortality up to the dose level of 5000 mg/kg b.wt in the treated animals were recorded daily during 14 days after the administration and hence it was considered safe for further pharmacological screening.

Experimental design

All the animals were acclimatized to laboratory conditions for 1 week before and fasted for 24 h prior to the experiment. The animals were divided into 6 groups (6 rats per each) as follows: Group I animals considered as control group. Group II animals were treated as gastric ulcer induced IND group. Injection of a single dose of 30 mg/ kg b.wt. (IND). Group III animals were pretreated with 50 mg/kg b.wt. of RAN. Group IV ulcer induced animals pretreated with 125 mg/kg b.wt. of IND and 250 mg/kg b.wt. of GPK algae extract. Group V animals concurrently pretreated with 500 mg/kg b.wt. GPK extract and RAN. Group VI animals were fed with 500 mg/kg b.wt. GPK algae extracts alone. Group III, IV, V, and VI run by orally two weeks before indomethacin administration immediately after pyloric ligation. Pyloric ligation was carried out in each animal before indomethacin administration to collect gastric juice.

Assessment of gastric mucosal lesions

All groups of animals were sacrificed three hours after indomethacin administration. Each stomach was removed and opened along the greater curvature, and the gastric juice was collected. The gastric mucosal lesions were expressed in terms of ulcer index (U.I.) which depends on the calculation of a lesion index by using about a 0-3 scoring system based on the severity of the each lesion according to their length (Peskar *et al*., 2002). Severity factor 0 = no lesions; 1 = lesion < 1 mm length; 2 = lesions 2-4 mm length and 3 = lesions > 4 mm length. The U.I. For each group was taken as the mean lesion score of all the rats in that group. The preventive index (P.I.) of a given drug was calculated by the equation of (Hano *et al*., 1976).

$$P.I. = \frac{\text{U.I. Of IND group - U.I. Of pretreated group}}{\text{U.I. Of IND group}} \times 100$$

Analysis of gastric juice

Gastric juice collected from each animal was centrifuged at 3000 RPM for 10 min to remove any solid debris. The volume of the supernatant was measured, then assayed for the pH[13] pepsin activity (Sanyal *et al*., 1971) and mucin concentration (Winzler, 1955). Free and total acid outputs were calculated by multiplying gastric juice volume by the measured free and total acid concentrations, respectively (Hara *et al.,* 1991; Feldman, 1998).

Biochemical analysis of gastric mucosa

The levels of MDA, NO and CAT was estimated by concern method (Mihara and Uchiyama, 1978; Sastry *et al*., 2002; Aebi, 1984). Prostaglandin E2 (PGE2) assay was performed with the PGE2 enzyme immunoassay kit.

Histopathological studies

The gastric mucosal section were dissected out and rinsed with ice-cold saline. A longitudinal section of gastric tissue was and fixed in a 10% formalin solution. After 24 hrs of fixation, tissues embedding in a paraffin block, then it was cut into sections of 5 microns onto a glass slide and stained with hematoxylin-eosin for histological assessment (Bancroft *et al*., 1996).

RESULTS AND DISCUSSION

Statistical analysis

All values are expressed as Mean ± SEM., and the Student's t-test were used to determine statistical evaluations were performed by ANOVA. Differences was considered to be significant when $p<0.05$. All analysis was made with the graph pad prism 5 statistical software.

Effect of GPK algae extracts on indomethacin induced gastric lesions

Ulcer index was significantly increased ($p < 0.01$) in the IND-treated group of animals (Group II) compared to normal animals (Group I).Treatment with ethanol extract of GPK showed a notable reduction ($p < 0.05$ and $p < 0.01$) (groups IV and V) in ulcer index compared to the IND- treated group (group II). However, GPK algae extract alone (group VI) did not show any remarkable effect on ulcer index.

Effect of GPK algae extracts on the gastric juice analysis

IND administration caused significant decrease in pH value and in gastric juice mucin content associated with remarkable increase in gastric juice free and total acidity and in gastric juice pepsin activity (compared to normal control group I). Pretreatment with RAN either alone or with GPK algae extract significantly increased the pH value and mucin contents whereas notable decrease in free and total acidity and gastric juice pepsin activity (compared to IND group II) indomethacin group). GPK algae extract alone (group VI) did not show any notable effect on Pepsin, and mucin levels. The ulceration induced by IND is attributed mainly to the various processes, including the generation of reactive oxygen species, initiation of lipid peroxidation, infiltration of the leukocytes, induction of the apoptosis, and inhibition of prostaglandin synthesis

(Bech *et al*., 2000). The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is considered to be the major risk factor in gastric ulcers. Oral administration of GPK algae extract produced significant drop in ulcerative index. Therefore, this result reinforced the possible strengthening of gastroprotective factors such as antioxidant elements in this extract.

Effect of GPK algae extracts on gastric mucosal lipid peroxides (MDA) & Catalase (CAT) activity

IND administration caused significantly raised the gastric mucosal MDA & CAT concentration observed in the control group (compared to group II). Treatment with GPK algae extract reduced the gastric mucosal MDA as well as CAT concentration. While, RAN pretreatment decreased the gastric mucosal MDA concentration. Mucosal damage can be easily produced by the generation of exogenous and endogenous active oxygen and free radicals (Naito *et al*., 1995). Indomethacin is known to induce the reactive oxygen metabolites in animal models, may contribute to mucosal injury (Chattopadhyay *et al*., 2006). This might lead to aggravated tissue damage during stomach ulceration (El Missiry *et al*., 2001). Ranitidine, an antisecretory drug, has often been reported to possess antioxidant and immunosuppressive actions, which may be responsible for its antiulcerogenic activity (Lapenna *et al*., 1994; Ardestani *et al*., 2004).

Effect of GPK algae extracts on the gastric mucosal nitrites/nitrate content

In indomethacin group, gastric mucosal nitrites/nitrate content was significantly reduced. Pretreatment of GPK algae extract significantly increased gastric mucosal nitrites/nitrate content (Group IV&V). Nitric oxide (NO) is an endogenous defensive factor for gastric cells and exhibits gastroprotective properties against different types of aggressive agents (Samini *et al*., 2002).

Effect of GPK algae extracts on the gastric mucosal prostaglandin E2 (PGE2) level

The synthesis of mucosal PGE2 was markedly arrested by indomethacin (compared to normal). However, the GPK algae extract- pretreated rats increased the PGE2 level marginally (compared to IND). Indomethacin causes ulcer mostly on the glandular (mucosal) part of the stomach (Nwafor *et al*., 1996) by inhibiting prostaglandin synthesis through the inhibition of the cyclooxygenase enzymes (Rainsford, 1987).

Effect of GPK algae extracts on serum level of Tumour necrosis factor α (TNFα)

Serum level of Tumour necrosis factor (TNFα) were obviously increased ($p < 0.01$) in the indomethacin treated group of animals (compared to normal) whereas the ethanol extract of the GPK algae treatment showed significant ($p < 0.05$ and $p < 0.01$) (groups III and IV) decrease in concentrations of serum TNFα (compared to IND group II). Tumour necrosis factor (TNF-α) is a proinflammatory cytokine secreted by macrophages increased during ulcerative stress (Hamaguchi *et al*., 2001), it is a potent stimulator of neutrophil infiltration into the gastric mucosa (Wei *et al*., 2003) and inducible nitric oxide expression (Calatayud *et al*., 2001).

Histopathological Examination

Histological examination of the gastric mucosal tissue showed sharply damaged mucosal epithelium, leukocyte infiltration and ulcerated area covered with inflammatory exudates were observed in aspirin induced rats. Treatment with GPK extract showed absence of ulcer crater, clearance of the necrosis and maintenance of the mucosal layers.

Conclusion

We conclude from the above results the ethanolic extract of Griffithsia pacifica kylin algae possessed significant gastroprotective and antioxidant potential. The findings of present study suggested that GPK could be potential natural source of antioxidant and supports in the treatment of gastrointestinal disorders.

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