IJP (2016), Vol. 3, Issue 3 **(Research Article)**

Received on 08 December 2015; received in revised form, 12 February 2016; accepted, 27 March 2016; published 31 March 2016

GASTRO PROTECTIVE EFFECTS OF *USNEA LONGISSIMA* METABOLITES ON PROBIOTIC *LACTOBACILLUS CASEI*

Lubna Azmi [1], Shyam Sundar Gupta [1], Ila Shukla [1], Padam Kant [2], D. K. Upreti [1] and Ch. V. Rao [*1]

Pharmacognosy and Ethnopharmacology Division [1], CSIR-National Botanical Research Institute, Lucknow - 226001, Uttar Pradesh, India.
Department of Chemistry [2], Lucknow University, Lucknow - 226007, Uttar Pradesh, India.

Keywords:

Probiotic, Gastric-ulcer, Lichen metabolites, *Lactobacillus casei*

Correspondence to Author:
Dr. Ch. V. Rao

Pharmacognosy and Ethnopharmacology Division, CSIR-National Botanical Research Institute, Lucknow - 226001, Uttar Pradesh, India.

E-mail: chvrao72@yahoo.com

ABSTRACT: Protective effect of the probiotic combination of usnic acid and *Lactobacillus casei* in experimentally induced ulcer in rats was investigated. Rats inward usnic acid (100 mg/kg), *L. casei* (10^{-8} con.), usnic acid (100 mg/kg) + *L. casei* (10^{-8} con.), and omeprazole (30 mg/kg) twice daily for 5 days for prevention against aspirin (ASP), ethanol (EtOH), cold restraint stress (CRS) and pylorus ligation-induced ulcer (PL). The results of the present study showed the first time that the usnic acid (100 mg/kg) + *L. casei* (10^{-8} con.) as probiotic combination significantly inhibited the ulcer index in ASP, EtOH, CRS and PL to 3.4 ± 0.12, 6.3 ± 1.8, 3.4 ± 0.8 and 4.3 ± 0.9 ($p<0.001$) respectively, as compared to control group (19.2 ± 1.6, 22.5 ± 6.3, 24.2 ± 3.2, 14.2 ± 2.7). Besides usnic acid (100 mg/kg) + *L. casei* (10^{-8} con.) offered protection (72%, $p<0.001$) against ethanol-induced depletion of gastric wall mucus. The usnic acid (100 mg/kg) + *L. casei* (10^{-8} con.) showed significant inhibition of lipid peroxidation and superoxide dismutase (5.46 ± 1.30, 120.6 ± 3.2) ($p<0.01$, $p<0.001$) respectively and enhance activity of catalase (32.2 ± 1.3, $p<0.001$) to healthy group range (34.2 ± 2.7, $p<0.001$). However, it elevated the decreased level of PGE_2 from 0.62 ± 0.12 ($p<0.05$) to 2.03 ± 0.51 ($p<0.001$) as compared to omeprazole. These results suggest that probiotic combination could attenuate the severity of gastric ulcer and prevent the toxicity level of usnic acid in the liver.

INTRODUCTION: Abnormal secretion of gastric acid and pepsin are the main reason for gastric ulcer disease. Nowadays, critical issue for gastric ulcer disease is gastric hyper secretion-linked with gastrinoma in Zollinger-Ellison syndrome, raise in parietal-cell mass, antral G-cell hyperplasia, and a physiological disproportion between the antagonistic gastric hormones gastrin and somatostatin.

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.IJP.3(3).140-48

Article can be accessed online on:
www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.3(3).140-48

It is known that cholinergic hypersensitivity and parasympathetic dominance both are related to pepsin [1]. Besides having anti-inflammatory and analgesic activity, NSAID especially aspires; significantly increase the risk of gastrointestinal infection in Asian countries. Individually those are related to gastric injury: Ulcer complications especially bleeding. NSAIDs cause an injurious effect by the inhibition of COX1 and its function in standard mucosal protection mechanisms, and also by the inhibition of thromboxane A_2, which accord platelet function and cause in gastrointestinal bleeding [2-5] but simultaneous low-doses of aspirin reduces the effect [2]. However, the interaction between microbial flora and pathogenic organisms has been reported [6].

Probiotics have been used to decrease the colonization of intestinal pathogens in human, and these are surviving microbial food ingredients that alter the enteric flora and have a constructive effect on health [7]. *L. casei* is probably the best-known probiotic micro-organism and its ability to hold stomach wall and to grow under acidic circumstances [8]. *L. casei* as supplement improve immunity and decrease the risk of disease akin to colon cancer, allergies, diarrhoea [9] and can prevent peptic ulcer which is caused by long term use of NSAIDs like aspirin.

Lichens are symbiotic associations of algae and fungi. Indeed, *Usnea longissima* (family Parmeliaceae with 1000 species across the world), has been used conventionally for pain relief and fever control [10]. It is also effective as anti-bacterial, anti-inflammatory, detoxifying agent [11]. Usnic acid **Fig. 1**, most common and abundant metabolite of *U. longissima* [12], has anti-viral, antiprotozoal, anti-inflammatory, anti-pyretic, anti-tumoral, liver protective and analgesic activity [13-17]. Indeed, the effect of *U. longissima* showed significant antiulcer activity.

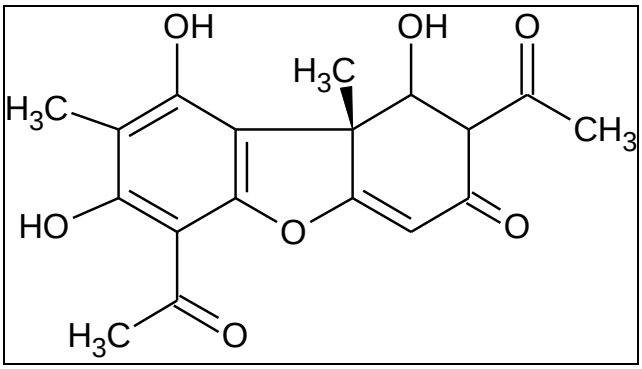

FIG. 1: STRUCTURE OF USNIC ACID

In continuation, the aim of our present study is to reduce the toxicity of usnic acid along with probiotic potential [17]. Therefore, in this study, we have investigated the anti-ulcer activity of *L. casei* in combination with lichen secondary metabolites.

MATERIALS AND METHODS:

Collection and Identification: Lichen material (*Usnea longissima*) was collected from Uttarakhand (India) in September 2012, known by its vernacular names *U. longissima*. The specimen has been submitted and perpetuate as the specimen in the herbarium of CSIR-National botanical research Institute, Lucknow (LWG).

Extraction and Isolation of Usnic Acid: Air-dried parts (200 g) of *U. longissima* were extracted with 300 ml of diethyl ether by Soxhlet apparatus, and usnic acid was isolated. Filtered extract was stored at 3-5 °C for 24 h to precipitate out usnic acid. A precipitate was subjected to silica gel (70-230 mesh) column chromatography eluted with chloroform and *n*-hexane (90:10). 876.5 mg of usnic acid was obtained with 0.88% (w/w) yield. UV, IR and NMR determined chemical structure of usnic acid and also confirmed by previously established spectral data [18].

General Analytical Procedures: All the required chemicals were purchased from Mark (India). UV–Visible spectrum for the biochemical assays of Usnic acid was taken by using lambda 25 PerkinElmer spectrophotometer. Alpha Bruker FT-IR spectrophotometer was used to record IR spectra, in KBr pellets. NMR spectra obtained at 200MHz for 1H and 50MHz for 13C (δ) on Varian spectrometer.

Animals: Experimental animals (Wistar rats of either sex) were acquired from the National Laboratory Animal Centre, Lucknow, India. For one week all the animals were kept under conventional conditions (temp., 24 ± 5 °C, and relative humidity 40-46%) and fed traditional rodent pellet diet (Amrut, India). Light/dark cycles of 12 h respectively, were maintained for seven days before and during the experiments. The food was withdrawn 18-24 h before the experiment while water was allowed impromptu. Experiments were performed according to the guidance given by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 173/GO/Re/13/ CPCSEA).

Microorganism: Bacterial strain *L. casei* (NCIM 2737) was procured from National Collection of Industrial Microorganisms at National Chemistry Laboratory, Pune, India and maintained on recommended MRS medium. To determine the suitable concentration of *L. casei* for the study of growth promoting effects of lichen metabolites, bacterial culture was serially diluted in the concentration of 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} in MRS broth medium and kept at 37 °C for 24 h. The resulting 10^{-8} concentration having $OD_{550} = 0.2$ was selected for the experiments

Doses: Usnic acid in dose of 100 mg/kg (p.o.), *L. casei* 10^{-8} con (p.o.), usnic acid 100 mg/kg (p.o.) + *L. casei* 10^{-8} con. (p.o.), and H^+ K^+ ATPase blocker, omeprazole in a dose of 30 mg/kg (i.p.) were administered twice daily at 09:00 and 15:00 h, respectively for 5 days for acute ulcer protective studies. Control group of animals received a suspension of 1% w/w carboxymethyl cellulose in double distilled water (10 ml/kg).

Aspirin-Induced Ulcers (ASP): Aspirin was administered at a dose of 200 mg/kg and ulcers were created after 4 h. Animals were sacrificed, and the abdomen was then excised [19]. It was cut on the larger curvature and washed thoroughly with 10.0 ml of 0.9% NaCl. Ulcers where scored by the person unaware of the experimental protocol. Lesion index was calculated after recording the full severity of ulcers per abdomen. Total severity of the ulcers resolved by the technique of Sanyal *et al.*, 1982. [20]

Ethanol-Induced Ulcers (EtOH): Gastric ulcers were induced in rats by administering ethanol (1ml/200 g, 1 h) [21]. After cervical dislocation of animals, the stomach was cut along the greater curvature and inspected for ulcers. Ulcer index (mm^2/ rat) was calculated by multiplying length and breadth of ulcers present in the glandular portion of the stomach. Unpaired Student's *t*-test was used for Statistical analysis.

Cold-Resistant Stress-Induced Ulcers (CRS): About 18 h before the experiment food was withdrawn, but the water was allowed. On the sixth day, experimental animals were immobilized by fastening the fore and hind limbs on a wooden board and left for 2 h, at 4-6 °C temperature [22]. After 2 h, the animals were sacrificed. The degree of lipid peroxidation (LPO) was also calculated under the stress condition using the standard method of Jamall and Smith, 1985. [23] Inhibition of the auto-oxidation of pyrogallol was taken as a measure of the activity of superoxide dismutase (SOD) [24]. Enzyme-catalyzed, the decomposition of hydrogen peroxide by potassium permanganate was used to determine CAT activity [25].

Pylorus–Ligation- Induced Ulcers (PL): For ulcer induction in rats by administration of drugs for a period of 5 days the last dose was given on the 6^{th} day. The rats were kept for 18 h fasting. Animals were anesthetized in the anesthetic chamber by using pentobarbitone 35 mg/kg (i.p.). pylorous ligation was done by the opening of the abdomen without causing any damage to the blood supply. With interrupted sutures stomach was replaced and the abdomen wall was closed in two layers. Animals were deprived of water during the post-operative period [26] after 4 h stomachs were dissected and cut open along the greater curvature. Contents were collected for estimation of biochemical parameters. The ulcers were scored as described under ASP- induced ulcers. Gastric secretion volume, pH and HCl concentration were measured.

Measurement of Ulcer Index: Aspirin model of rats was used to create the ulcers. These were scored according to the arbiter scoring system and graded as Shedding of epithelium = 10; petechial and frank hemorrhages = 20; one or two ulcers = 30; more than two ulcers = 40; and perforated ulcers = 50. Area of ulcer base was measured by the help of biovis image analysis software in the ulcer model, which is considered as the ulcer index. Ulcer index is calculated from scorings described as under:

$$UI = Us + Up \times 10^{-1}$$

Where, Us is the severity of ulcer score; Up is the percentage of animals with ulcer incidence.

For anti-ulcer studies Percentage protection index is calculated as under:

$$C\text{-}T / C \times 100$$

Where, C is ulcer index for the control group; T is ulcer index for the treated group.

Determination of Gastric Wall Mucus: Gastric wall mucus was determined according to the method of Mizui *et al*., 1983. [27] The glandular segments from stomachs were removed, weighed and incubated in tubes containing 1% alcian blue solution (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8) for 2 h. The alcian blue binding extract was centrifuged at 3000 rpm for 10 min, and the absorbency of the supernatant was measured at 498 nm. The quantity of alcian blue extracted (gram per gram of glandular tissue) was then calculated.

Lipid Peroxidation (LPO): The thiobarbituric acid test was used to determine the concentration of gastric mucosal LPO by estimating malondialdehyde (MDA) [28]. After that, rat stomachs were excised without delay and washed with cold saline. The mucosa was cleansed adequately of the blood, to reduce the interference of hemoglobin with free radicals. The corpus mucosa was scratch out, weighed and homogenized in 10mL of 100 g/l KCl. The homogenate (0.5 ml) was mixed with 1.5 ml of 8 g/l 2-thiobarbiturate, 1.5 ml of 200 g/l acetic acid, 0.2 ml of 80 g/l sodium lauryl sulfate and 0.3 ml distilled water. The mixture was incubated at 98^0C for 1 h. This mixture was cooled and 5 ml of *n*-butanol: pyridine (15:l) was added to it. First the mixture vortexed for 1 min and then centrifuged for 30 min at 4000 rpm. Supernatant was collected and absorbance was measured at 532 nm. The standard curve was constructed by using 1,1,3,3-tetramethoxypropane. The recovery was about 90%. The results were expressed as nanomole MDA per gram of wet tissue (nmol g tissue^{-1}).

Superoxide Dismutase Activity (SOD): SOD activity was measured according to Sun *et al.*, 1988. [29] For SOD estimation xanthine and xanthine oxidase activity was measured based on the generation of superoxide radicals. These super-oxide radicals react with nitro blue tetrazolium (NBT) to form formazan dye. The degree of inhibition of the reaction at 560 nm was taken as a measure of SOD activity and was expressed as millimole per minute per milligram tissue (mmol min^{-1} mg tissue^{-1}).

Catalase Activity (CAT): Catalase decomposed H_2O_2 and its activity were recorded at 240 nm [25]. CAT activity is defined as the amount of enzyme needed to decompose 1 nmol of H_2O_2 per minute, at 25^0C and pH 7.8. Results were expressed as millimole per minute per milligram tissue (mmol min^{-1} mg tissue^{-1}).

Estimation of Mucosal PGE_2: Frozen gastric mucosal tissue (1 g) was added to 5 ml homogenization buffer 0.1 M phosphate (pH 7.4), containing 1 mM EDTA and 10 mM indomethacin. The lysate was then centrifuged in a microcentrifuge at 16,000 × g for 15 min at 2 °C to 8°C. The supernatant was transferred to a new tube, and total protein content was analyzed using the sophisticated protein assay. PGE_2 concentrations were investigated using the PGE_2 ELISA Kit CS0200S (Sigma Aldrich) [30].

Statistical Analysis: All the results were presented as mean ± SEM for six rats and analyzed by Wilcoxon Sum Rank Test and unpaired Student's *t*-test used for determination of the level of significance between various groups. Value of $p< 0.05$, 0.01 and 0.001 was considered statistically significant.

RESULTS: Usnic acid showed significant ulcer protective effect, and it showed better positive effect when it was given with *L. casei* twice daily for 5 days against aspirin (ASP), ethanol (EtOH), cold restraint stress (CRS) and pyloric ligation (PL) induced gastric ulcer. Usnic acid supplemented *L. casei* showed significant ($p<0.001$) decrease in ulcer index (UI) against ulcers induced by: (1) ASP (control UI: 19 ± 1.6), % curative ratio levels of *L. casei*, usnic acid, omeprazole, usnic acid + *L. casei* are 23.95%, 70.8%, 67.7% and 82.3% respectively ($p<0.05$ to $p<0.001$); (2) EtOH (control UI: 22.5 ± 6.3), % curative ratio levels of *L.casei*, usnic acid, omeprazole, usnic acid + *L. casei* 20.8%, 67.11%, 59.55% and 72% respectively ($p<0.05$ to $p<0.001$); (3) CRS (control UI: 24.2 ± 3.2), % curative ratio levels *L. casei*, usnic acid, omeprazole, usnic acid + *L. casei* 17.35%, 79.75%, 80.99% and 85.95% respectively ($p<0.05$ to $p<0.001$); (4) PL (control UI: 14.2 ± 2.7), % curative ratio levels *L. casei*, usnic acid, omeprazole, usnic acid + *L. casei* 28.16%, 63.38%, 59.83% and 69.17% respectively ($p<0.05$ to $p<0.001$) **Table 1**.

Secretion of mucus and bicarbonate by the surface epithelial constitute mucus–bicarbonate barrier, which is regarded as the first line of defense against potential ulcerogenic. The gastric wall mucus was significantly enhanced (UI: 225.7 ± 14.6, % curative ratio: 72%, $p<0.001$) by probiotic combination of usnic acid + *L. casei* as compared to usnic acid 100 mg/kg (UI:199.1 ± 8.3, % curative ratio: 67%, $p<0.01$) and omeprazole 30 mg/kg (UI: 213 ± 10.1, % curative ratio: 60%, $p<0.001$) administered twice daily and is regarded as a first line of defense against EtOH-induced gastric ulcers showing cytoprotective property **Table 2**.

TABLE 1: EFFECT OF USNIC ACID AND *L. CASEI* ON ASPIRIN (ASP), ETHANOL (EtOH), COLD- RESTRAINT STRESS (CRS) AND PYLORUS LIGATION (PL) INDUCED ULCER

Treatment	Ulcer index			
	ASP	EtOH (mm^2/rat)	CRS	PL
Healthy Group	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
Control	19.2 ± 1.6	22.5 ± 6.3	24.2 ± 3.2	14.2 ± 2.7
Usnic acid	5.6 ± 0.35**	7.4 ± 2.1***	4.9 ± 1.3*	5.2 ± 1.3**
L. casei	14.6 ± 1.2	17.8 ± 4	20.0 ± 2.7	10.2 ± 2.3
Usnic acid + *L. Casei*	3.4 ± 0.12***	6.3 ± 1.8**	3.4 ± 0 .8***	4.3 ± 0.9***
Omeprazole	6.2±0.54***	9.1 ± 2.4***	4.6 ± 0.9 ***	5.7 ± 1.7***

Data are represented as mean ± S.E.M. for six rats. *p<0.05 ,**p<0.01 ,***p<0.001 compared to respective control group

TABLE 2: EFFECT OF USNIC ACID AND *L. CASEI* ON ETHANOL-INDUCED ULCER (EtOH) AND GASTRIC WALL MUCUS IN RATS

Treatment	EtOH (mm^2/rat)		
	Ulcer Index	Percent protection	(Gastric wall mucus gram per gram wet glandular tissue)
Healthy Group	0.0 ± 0	-	279.8 ± 16.3*
Control	22.5 ± 6.3*	-	171.6 ± 12.7*
Usnic acid	7.4 ± 2.1*	67%	199.1 ± 8.3**
L. casei	17.8 ± 4.1*	21%	177.2 ± 13.1**
Usnic acid + *L. Casei*	6.3 ± 1.8*	72%	225.7 ± 14.6***
Omeprazole	9.1 ± 2.4*	60%	213.6 ± 10.1***

Data are represented as mean ± S.E.M. for six rats. *p<0.05 ,**p<0.01,***p<0.001 compared to respective control group.

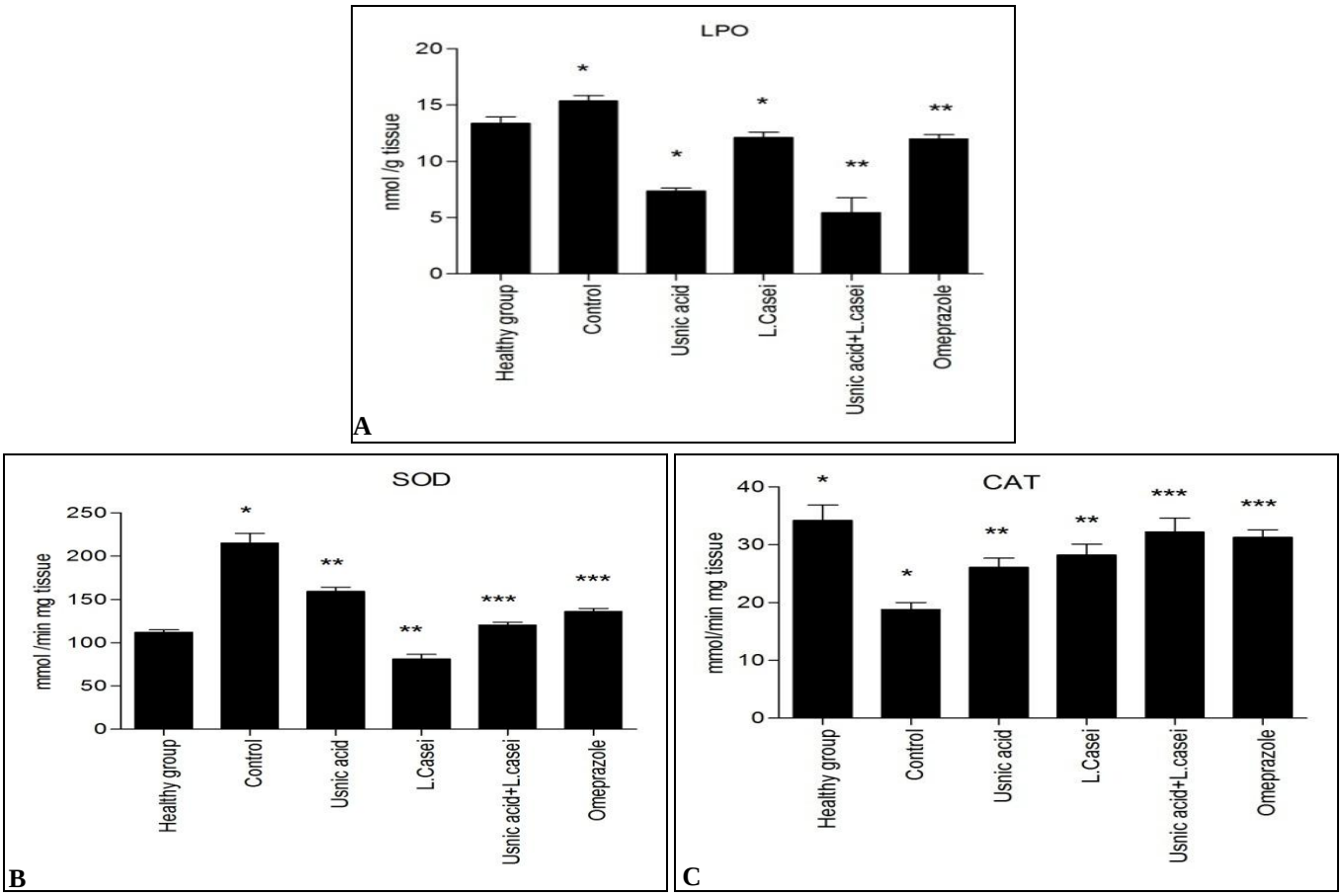

FIG. 2: EFFECT OF USNIC ACID AND *L. CASEI* ON (A) LIPID PEROXIDATION LPO, (B) SUPEROXIDE DISMUTASE SOD, (C) CATALASE CAT IN COLD-RESTRAINT STRESS (CRS)-INDUCED ULCER. Data are represented as Mean ± S.E.M. for six rats. *****p<0.05, **p<0.01, ***p<0.001 compared to respective control group.

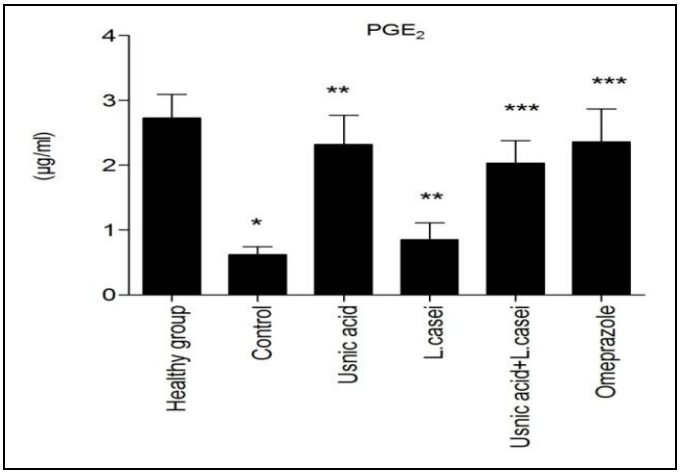

FIG. 3: EFFECT OF USNIC ACID AND *L. CASEI* ON PGE_2. Data is represented as Mean ± S.E.M. for six rats. *p<0.05, **p<0.01, ***p<0.001 compared to respective control group.

Cold-restraint stress significantly caused ulceration with concomitant increase in LPO (healthy group 13.4 ± 0.56, stress 15.37 ± 0.48 nmol g $tissue^{-1}$, p<0.05) and SOD (healthy group 112.10 ± 0.35, stress 215 ± 11.3 nmol g $tissue^{-1}$, p<0.05) and decrease in CAT (healthy group 34.2 ± 2.7, stress 18.8 ± 1.2 nmol g $tissue^{-1}$, p<0.05). When the animals were treated with usnic acid + *L. casei* there is significant reversal the ulcer index, LPO, SOD, and CAT levels near to normal values when compared to the stress group (LPO 5.46 ± 1.30, p<0.01; SOD 120.6 ± 3.2, p<0.001, CAT 32.2 ± 2.4, p<0.001) **Fig. 2**. There was increase in PGE_2 (control: 0.62 ± 0.12, p<0.05 to 2.03 ± 0.35, p<0.001) when usnic acid + *L. casei* administered **Fig. 3**.

DISCUSSION: Peptic ulcer disease is the result of an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defense mechanism. In the present study, we have investigated the effect of lactobacillus in combination with usnic acid on peptic ulcer disease -synthetic NSAIDs like aspirin cause mucosal damage by inhibition of COX enzyme.

COX (cyclo-oxygenase) enzyme comprises COX-1 and COX-2. COX-2 is especially linked to inflammation. Aspirin is an example of classical NSAIDs; it inhibits both COX-1 and COX-2 enzymes. COX-1 is responsible for sustaining baseline levels for prostaglandins (PgE_2), responsible for maintaining the defense mechanism in the stomach against ulcerogenic content [31-32]. The combination of usnic acid and *L. casei*, act as a protectant against ulcer-causing substances by increasing the thickness of the mucosal barrier. Mucosal barrier thickened as *L. casei* increased the expression of mucin and levels of PgE_2, which simultaneously acted to enhance the levels of mucous secretion in the stomach. Ethanol-induced ulcers are more common in the glandular part of stomach. Ethanol is reported to initiate the synthesis of leukotriene C4 (LTC4), mast cell secretory products [33] and reactive oxygen species (ROS), which causes damage to gastric mucosa [34].

Combination of usnic acid and *L. casei* increased the levels of prostaglandins which in turn led to an increase in mucous secretion. A profuse amount of gastric mucus is secreted during the superficial mucosal damage which creates a favourable microenvironment for restoration for the injury. Ethanol-induced depletion of gastric wall mucous was significantly prevented by the given combination.

Stress plays an essential role in the pathology of gastro-duodenal ulceration. A stress-induced ulcer is possibly mediated by histamine release which reduces the production of mucus. Other factors leading to stress-induced ulcers are a decrease in gastric mucosal blood flow [35], decreased synthesis of prostaglandins [36], an increase in gastric motility, increased vagal activity [37]. Accordingly, the protective action of the given combination against the cold stress could be due to an increase in mucin expression, suppression of bacterial overgrowth, the rise in the synthesis of antioxidants and stimulation of mucosal immunity.

Oxidative damage is a frequently reported cause in the pathogenesis of ulcer by different experimental and clinical models. A stress-induced ulcer is due to an increase in the generation of free radicals, apart from acid pepsin factor [38]. Stress significantly initiate lipid peroxidation as it is clear from the increased levels of LPO. This was the result of an enhanced generation of reactive oxygen species due to stress. Generally, the damage due to ROS is contained by dismutation with SOD, which converts reactive oxygen to H_2O_2 [39]. If the CAT does not scavenge it, it can cause lipid peroxidation by an increase in the generation of hydroxyl radicals [40]. Therefore a decrease in CAT levels favored the accumulation of these reactive oxygen species and hence, caused the increase in lipid peroxidation and tissue damage.

The effect is further worsened by the alleviated activity of gastric peroxidases during stress. Treatment by the combination of usnic acid and *L. casei* reversed the oxidative damage caused by stress. Pyloric ligation caused the accumulation of gastric acid and pepsin, leading to autodigestion of gastric mucosa [41]. When combination was administered; it increased the synthesis of prostaglandins leading to enhanced production of mucous which maintained the thickness of the gastric mucosal wall. Increase in the levels of PgE_2 after the administration of the combination reveals that this given combination protects the gastric wall mucosa by enhancing the synthesis of prostaglandins which in turn increases the production of mucus.

ACKNOWLEDGEMENT: This research supported in part with funds from the Science and Engineering Research Board (SERB/F/3970/2014-15), New Delhi. Authors are thankful to the Director, CSIR-National Botanical Research Institute, Lucknow, India for providing necessary facilities.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Yuan Y and Hunt RH: Treatment of non-NSAID and non-*H. pylori* gastroduodenal ulcers and hypersecretory states. In: Wolfe M, ed. Therapy of digestive disorders, Philadelphia, PA: Saunders Elsevier; Edition 2nd, 2006: 315-336.
2. Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM, Zhao WW, Kent JD, Lefkowith JB, Verburg KM and Geis GS: Gastrointestinal toxicity with celecoxib *vs.* Non-steroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the class study. A randomized controlled trial. Celecoxib long-term arthritis safety study. Journal of the American Medical Association 2000; 284(10): 1247-1255. doi: 10.1001/jama.284.10.1247 PMID: 10979111
3. Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK Schnitzer TJ and Vigor: Study Group: Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. The New England journal of medicine 2000; 343(21): 1520-1528. doi: 10.1056/NEJM200011233 432103 PMID: 11087881
4. Hunt RH, Harper S, Watson DJ, Yu C, Quan H, Lee M, Evans JK and Oxenius B: The gastrointestinal safety of the COX-2 selective inhibitor etoricoxib assessed by both endoscopy and analysis of upper gastrointestinal events. The American Journal of Gastroenterology 2003; 98(8):1725-1733. doi: 10.1111/j.1572-0241.2003.07598.x PMID: 12907325
5. Schnitzer TJ, Burmester GR, Mysler E, Hochberg MC, Doherty M, Ehrsam E, Gitton X, Krammer G, Mellein B, Matchaba P, Gimona A and Hawkey CJ: Target Study Group: Comparison of lumiracoxib with naproxen and ibuprofen in the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET) reduction in ulcer complications randomised controlled trial. Lancet 2004; 364(9435): 665-674. doi: 10.1016/S0140-6736(04)16893-1 PMID: 15325831
6. Reid G, Bruce AW, Mcgroarty JA, Cheng KJ and Costerton JW: Is there a role for lactobacilli in prevention of urogenital and intestinal infections? Clinical Microbiology Reviews 1990; 3(4): 335-344. PMCID: PMC358167
7. Sanders ME and Huis isn't Veld J: Bringing a probiotic-containing functional food to the market: Microbiological, product, regulatory and labelling issues. Antonie van Leeuwenhoek 1999; 76(1): 293-315. PMID: 10532385
8. Naidu AS, Bidlack WR and Clemens RA: Probiotic spectra of lactic acid bacteria (LAB). Critical Reviews in Food Science and Nutrition 1999; 39(1):13-126. doi: 10.1080/10408699991279187 PMID: 10028126
9. Grajek W, Olejnik A and Sip A: Probiotics, prebiotics and antioxidants as functional foods. Acta Biochimica Polonica 2005; 52(3): 665-671.
10. Okuyama E, Umeyama K, Yamazaki M, Kinoshita Y and Yamamoto Y: Usnic acid and diffractaic acid as analgesic and antipyretic components of *Usnea diffracta*. Planta medica 1995; 61(2):113-115. doi: 10.1055/s-2006-958027 PMID: 7753915
11. Lauterwein M, Oethinger M, Belsner K, Peters T and Marre R: *In-vitro* activities of the lichen secondary metabolites vulpinic acid, (+)-usnic acid, and (-)-usnic acid against aerobic and anaerobic microorganisms.

Antimicrobial Agents and Chemotherapy 1995; 39(11): 2541-2543. doi: 10.1128/AAC.39.11.2541 PMCID: PMC162980

12. Mallavadhani UV, Sudhakar AVS, Mahapatra A, Narasimhan K, Thirunavokkarasu M and Elix JA: Phenolic and steroidal constituents of the lichen *Usnea longissima*. Biochemical Systematics and Ecology 2004; 32(1): 95-97. doi: 10.1016/S0305-1978(03)00187-X
13. Ingolfsdottir K, Chung GAC, Skulason VG, Gissurarson SR and Vilhelmsdottir M: Antimycobacterial activity of lichens metabolites *in-vitro*. European Journal of Pharmaceutical Sciences 1998; 6(2):141–144. doi: 10.1016/S0928-0987(97)00078-X PMID: 9795033
14. Fournet A, Ferreira ME, Arias ARD, Ortiz ATD, Inchausti A, Yaluff G, Quilhot W, Fernandez E and Hidalgo ME: Activity of compounds isolated from Chilean lichens against experimental cutaneous leishmaniasis. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 1997; 116(1): 51-54. doi: 10.1016/S0742-8413(96)00127-2 PMID: 9080673
15. Kupchan SM and Kopperman HI: L-usnic acid: tumor inhibitor isolated from lichens. Experientia 1975; 31(6):625-626. doi: 10.1007/BF01944592. PMID: 124660
16. Vijayakumar CS, Viswanathan S, Kannappa-Reddy M, Parvathavarthini S, Kundu SB and Sukumar E: Anti-inflammatory activity of (+) usnic acid. Fitoterapia 2000; 71(5):564-566. doi: 10.1016/S0367-326X(00)00209-4
17. Pramyothin P, Janthasoot W, Pongnimitprasert N, Phrukudom S and Ruangrungsi N: Hepatotoxic effect of (+)-usnic acid from *Usnea siamensis* Wainio in rats, isolated rat hepatocytes and isolated rat liver mitochondria. Journal of Ethnopharmacology 2004; 90(2-3):381-387. doi: 10.1016/j.jep.2003.10.019
18. Huneck S and Yoshimura I: Identification of Lichen Substances. Springer, Berlin, Heidelberg, 1996: 493.
19. Goel RK, Das DG and Sanyal AK: Effect of vegetable banana powder on changes induced by ulcerogenic agents on dissolved mucosubstances in gastric juice. Indian Journal of Gastroenterology 1985; 4(4): 249-251. PMID: 3850848
20. Sanyal AK, Pandey BL and Goel RK: The effect of a traditional preparation of copper, tamrabhasma, on the experimental ulcer and gastric secretion. Journal of Ethnopharmacol 1982; 5(1): 79-89. doi: 10.1016/0378-8741(82)90023-X PMID: 7054601
21. Hollander D, Tarnawski A, Krause WJ and Gergely H: Protective effect of sucralfate against alcohol-induced gastric mucosal injury in the rat. Macroscopic, histologic, ultrastructural, and functional time sequence analysis. Gastroenterology 1985; 88 (1 Pt 2): 366-374. doi: 10.1016/S0016-5085(85)80191-8 PMID: 3871090
22. Gupta MB, Nath R, Gupta GP and Bhargava KP: A study of the antiulcer activity of diazepam and other tranquillo-sedatives in Albino rats. Clinical and experimental pharmacology & physiology 1985; 12(1):61-66. doi: 10.1111/j.1440-1681.1985.tb00303.x
23. Jamall IS and Smith JC: Effects of cadmium on glutathione peroxidase, superoxide dismutase, and lipid peroxidation in the rat heart: A possible mechanism of cadmium cardiotoxicity. Toxicology and Applied Pharmacology 1985; 80(1):33-42. doi: 10.1016/0041-008X(85)90098-5
24. Marklund S and Marklund G: Involvement of superoxide anion radical and a convenient assay of superoxide dismutase. European Journal of Biochemistry 1974; 47(3): 469-474. doi: 10.1111/j.1432-1033.1974.tb03714.x PMID: 4215654
25. Beers RF Jr and Sizer IW: A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. The Journal of Biological Chemistry 1952; 195(1): 133-140. PMID 14938361
26. Shay H, Komarov SA, Fels SS, Meranze D, Gruentein M and Siplet H: A simple method for the uniform production of gstric ulceration. Gastroenterology 1945; 5: 43-61.
27. Mizui T, Doteuchi M: Effect of polyamines on acidified ethanol-induced gastric lesions in rats. Japanese Journal of Pharmacology 1983; 33(5):939-945. doi: 10.1254/jjp.33.939 PMID 6580476
28. Ohkawa H, Ohishi N and Yagi K: Assay for lipid peroxides in animal tissue by the thiobarbituric acid reaction. Analytical Biochemistry 1979; 95(2): 351-358. DOI: 10.1016/0003-2697(79)90738-3 PMID: 36810
29. Sun Y, Oberley LW and Li Y: A simple method for clinical assay of superoxide dismutase. Clinical chemistry 1988; 34(3): 497-500. PMID: 3349599
30. Wang Z, Hasegawa J, Wang X, Matsuda A, Tokuda T, Miura N and Watanabe T: Protective effects of ginger against aspirin-induced gastric ulcers in rats. Yonago Acta medica 2011; 54(1): 11-19. PMCID: PMC3763798
31. Matsui H, Shimokawa O, Kaneko T, Nagano Y, Rai K and Hyodo I: The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine. Journal of Clinical Biochemistry and Nutrition 2011; 48(2): 107-111. doi: 10.3164/jcbn.10-79 PMCID: PMC3045681
32. Rao ChV, Ojha SK, Radhakrishnan K, Govindarajan R, Rastogi S, Mehrotra S and Pushpangadan P: Antiulcer activity of *Utleria salicifolia* rhizome extract. Journal of Ethnopharmacology 2004; 91(2-3): 243-249. doi: 10.1016/j.jep.2003.12.020 PMID: 15120446
33. Oates PJ and Hakkinen JP: Studies on the mechanism of ethanol-induced gastric damage in rats. Gastroenterology 1988; 94(1): 10-21. PMID: 3335281
34. Mizui T, Sato H, Hirose F and Doteuchi M: Effect of antiperoxidative drugs on gastric damage induced by ethanol in rats. Life sciences 1987; 41(6): 755-763. doi: 10.1016/0024-3205(87)90456-5 PMID: 3613839
35. Hase T and Moss BJ: Microvascular changes of gastric mucosa in the development of stress ulcer in rats. Gastroenterology 1973; 65(2): 224-234. PMID: 4720026
36. Rao CV, Maiti RN and Goel RK: Effect of mild irritant on gastric mucosal offensive and defensive factors. Indian Journal of Physiology and Pharmacology 2000; 44(2): 185-191. PMID: 10846633
37. Cho CH, Ogle CW and Dai S: Acute gastric ulcer formation in response to electrical vagal stimulation in rats. European Journal of Pharmacology 1976; 35(1):215-219. doi: 10.1016/0014-2999(76)90319-8 PMID: 1253823

38. Miller T A: Mechanisms of stress-related mucosal damage. The American Journal of Medicine 1987; 83(6A): 8-14. doi: 10.1016/0002-9343(87)90805-9 PMID: 3321980
39. Fridovich I: Biological effects of the superoxide radical. Archives of biochemistry and biophysics 1986; 247(1): 1-11. doi: 10.1016/0003-9861(86)90526-6 PMID: 3010872
40. Das D, Bandyopadhyay D, Bhattacharjee M and Banerjee RK: Hydroxyl radical is the major causative factor in stress-induced gastric ulceration. Free radical biology & medicine 1997; 23(1): 8-18. doi: 10.1016/S0891-5849(96)00547-3 PMID: 9165292
41. Boyd SC, Sasame HA and Boyd MR: Gastric glutathione depletion and acute ulcerogenesis by diethylmaleate gave subcutaneously to rats. Life sciences 1981; 28(26): 2987-2992. doi: 10.1016/0024-3205(81)90276-9 PMID: 7266 259.

How to cite this article:
Azmi L, Gupta SS, Shukla I, Kant P, Upreti DK and Rao CV: Gastro protective effects of *Usnea longissima* metabolites on probiotic *Lactobacillus casei*. Int J Pharmacognosy 2016; 3(3): 140-48. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.3(3).140-48.