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Vanillin abrogates ethanol induced gastric injury in rats via modulation of gastric secretion, oxidative stress and inflammation

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ABSTRACT

Vanillin is commonly used as an additive in food, medicine and cosmetics, but its effect has not yet been studied in gastric injury. Therefore the effect of vanillin was studied in experimental gastric ulcer. Gastric secretion and acidity were studied in pylorus ligated rats. Ulcer index, levels of gastric mucus, malondialdehyde (MDA), myeloperoxidase activity (MPO), expression of nuclear factor kappa B (NF-κB) p65, and histopathological changes were determined in ethanol induced gastric ulcer. Pre treatment with vanillin significantly reduced gastric secretion ($P < 0.001$) and acidity ($P < 0.0001$) and gastric ulcer index scores ($P < 0.001$). and augmented the gastric mucosal defense. Vanillin significantly restored the depleted gastric wall mucus levels ($P < 0.0001$) induced by ethanol and also significantly attenuated ethanol induced inflammation and oxidative stress by the suppression of gastric MPO activity ($P < 0.001$), reducing the expression of NF-κB p65 and the increased MDA levels ($P < 0.001$). Vanillin was also effective in alleviating the damage to the histological architecture and the activation of mast cells induced by ethanol.

Together the results of this study highlight the gastroprotective activity of vanillin in gastric ulcers of rats through multiple actions that include inhibition of gastric secretion and acidity, reduction of inflammation and oxidative stress, suppression of expression of NF-κB, and restoration of the histological architecture.

1. Introduction

Gastric ulcer commonly characterized by an interruption of the mucosal integrity is a common and chronic disease in the adult population. Approximately 5–10% of people are affected by this disease during their life time [47]. A number of factors have been linked to the genesis of gastric ulcers that include malnutrition, helicobacter pylori infection, use of alcohol, anti-inflammatory drugs, stress, trauma, and burns [1,42,52,56]. Another important factor involved in the formation of gastric ulcers is the disruption of the homeostasis between the defensive (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) and the offensive factors (increased gastric secretion of pepsin and acid, helicobacter pylori) [56] in the gastric mucosa.

Gastric ulcer formation may also be due to disturbances in the microcirculation [55] leading to ischemia ensuing in the temporary loss or reduction in supply of blood and oxygen to the gastric mucosa. Restoration of the blood supply through reperfusion restores the function but may also initiate adverse events such as generation of free radicals (ROS), and inflammation at the site of injury. Infiltration of activated neutrophils is also a vital component in the formation of gastric ulcers. Excessive infiltration of neutrophils is indicated by an increase in the activity of the enzyme myeloperoxidase, which is used as a marker in gastric ulcer pathogenesis [7].

Oxidative stress [49] infiltration of neutrophils, over expression of inflammatory agents and generation of pro inflammatory cytokines [7,12] have all been shown to be involved in the pathogenesis of gastric ulcers. On the other hand the mucus layer, and endogenous antioxidants; components of the gastrointestinal defense help in the protection against ROS induced cytotoxicity [7,49]. Consequently reducing oxidative stress, inflammation and

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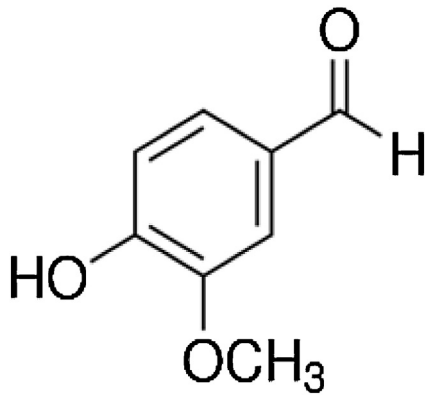

Fig. 1. Structure of Vanillin.

activation of neutrophils and strengthening of the gastric mucosal defense may be helpful against gastric damage by ulcerogens.

A number of drugs are available for the treatment of gastric ulceration; the common approach for the management of gastric ulcers is based on decreasing gastric secretion through H_receptor antagonists and proton pump inhibitors [19]. However the continuous and prolong use of these drugs may result in serious adverse effects [50], thus necessitating a constant search for new anti ulcer therapies. Since gastric ulcerogenesis is a multi etiologic disease, identification of drug/s agents that possess natural antiulcer properties such as reduction of gastric secretion, and inflammation, and an ability to strengthen the endogenous defensive capacity of the gastric mucosa may be helpful in the amelioration of gastric ulcers.

Dietary consumption and use of polyphenols is helpful in protecting against different diseases [44]. Vanillin (VLN) a *o*-methyl catechol (4-hydroxy-3-methoxybenzaldehyde) polyphenol is a flavor compound extracted from vanilla beans, and widely used as an additive in food, medicine and perfume [18], with an estimated annual consumption of more than 2000 tons all over the world [57] (Fig. 1).

Vanillin has been reported to possess anti inflammatory [24], anti colitis [59,22], antimutagenic [17], antioxidant and antitumor properties [15,34]. Furthermore VLN also ameliorates depression like behaviors in rats [61], and has a preventive effect in animal model of Huntington's disease through the reduction of oxidative and nitrosative stress [15]. In addition vanillin was also reported to reduce the expression of proinflammatory cytokines interleukin 1L 1β, IL-6, interferon-γ and tumor necrosis factor in colonic tissues of mice subjected to inflammatory bowel disease [60].

Keeping in view the strong antioxidant and potent anti inflammatory properties of vanillin we hypothesized that vanillin may provide protection against chemically induced gastric ulcers in rats. At the same time a thorough review of literature showed that the effect of vanillin has not been studied against ethanol induced gastric ulcers in rats. Therefore the present study investigates the gastroprotective potential of vanillin against ethanol induced gastric ulcers in rats.

2. Methods

The study protocol was approved by the institutional research and ethics committee. The institutional guidelines for the maintenance and use of animals were strictly followed. Male Wistar rats, weighing 200–225 g maintained at 12 h light/dark cycles and provided a standard chow diet and water ad libitum were used in all the experiments. The animals were divided into groups of six animals each. The aqueous solution of vanillin in doses of 50, 100 and 200 mg/kg body weight was freshly prepared and administered orally by gavage for both gastric secretion and antiulcer studies to different groups. The concentrations of drug were prepared in such a way that each rat received 0.5 ml of drug solution/100 g body weight.

2.1. Gastric secretion study

Pylorus Ligated (Shay) Rats: Pylorus ligation was performed under anesthesia in 36 h fasted rats with ad libitum access to water as per the method of Shay [43]. All essential precautions were taken to prevent bleeding or to occlude blood vessels. Vanillin in the desired doses was administered immediately after the ligation in three groups. One group served as control and received only water in the same volume as that of vanillin treated groups. The rats were killed six hours after the ligation and the stomachs were removed, The stomachs were emptied and the volume was measured for gastric secretion and centrifuged and analyzed for titratable acidity against 0.01 N NaOH to pH 7 using a pH meter and total acid output was calculated.

2.2. Induction of gastric ulcers

Gastric Lesions Induced by Ethanol (Cytoprotection Studies): Gastric ulcers were induced in rats by the administration (i.g.) of 1 ml of absolute ethanol as per the method of Natale et al. [30]. Vanillin in different doses was given (i.g.) 30 min before the administration of ethanol. The animals in the control and drug alone groups did not receive any ethanol. One hour after the administration of ethanol the animals in the different groups were killed and examined for the lesions in stomachs.

Scoring of Lesions: The method as described by Schiantarelli [39] was used for scoring the ethanol induced patched lesions of the stomach.

2.3. Histological and biochemical studies

Separate batches of animals (with six animals in each group) were used for biochemical and histological studies. The same protocol of ethanol and vanillin administration as mentioned above for gastric lesions was followed for these studies. Each batch of animals was comprised of six groups. Group one was considered as control and did not receive any ethanol. Group two was given ethanol, group three, four and five received vanillin in different doses 30 min before the administration of ethanol. Group six was drug alone control group and was administered the highest dose of vanillin only. Stomachs were collected after killing the animals at one hour after the administration of ethanol and assays were performed for gastric wall mucus, myeloperoxidase (MPO) activity, and malondialdehyde (MDA) levels. For histological studies stomach was preserved in neutral buffered formaldehyde.

2.4. Determination of gastric wall mucus

The procedure as described by Corne et al. [11] was used for the determination of gastric wall mucus.

2.5. Determination of malondialdehyde

Malondialdehyde (MDA) concentration a determinant of lipid peroxidation was measured in the gastric mucosa by the thiobarbituric acid test as per the method of Ohkawa et al. [32].

2.6. Determination of MPO

MPO activity in the gastric mucosa was measured according to the method described by Bradley et al. [9].