Original Article

Application of piper betle as an antioxidant against nonsteroidal anti-inflammatory drug-induced gastric ulcer

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Abstract: The present study was aimed to investigate the protective effect of Piper betle against nonsteroidal anti-inflammatory drug (NSAID)-induced gastric ulcer in the male albino rats. Piper betle (0, 50, 100 and 200 mg/kg bwt) was given for 15 consecutive days. Lipid peroxidation, catalase, superoxide dismutase (SOD), lactate dehydrogenase (LDH), reactive oxygen species (ROS) were determined. The above mentioned biochemical markers were increased in the drug-induced ulcer rats. Administration of Piper betle significantly reversed malondialdehyde (MDA), catalase, SOD, LDH and ROS towards a normal level. Caspase-3 expression was reduced in the control rats, whereas it was significantly increased following Piper betle administration. Taking all these data together, it may be suggested that Piper betle could be a potent therapeutic agent for treating gastric ulcers in the NSAID-induced gastric ulcer model in male albino rats.

Keywords: Piper betle, gastric ulcer, antioxidant, NSAID, rats

Introduction

Piper betle Linn is a well-known and widely growing plant in South East Asia, and it has been reported to act as a potential therapeutic agent for digestion and tumors [1]. Several researchers have reported the antifungal, antimicrobial and anti-inflammatory activity of Piper betle [2]. It contains several active constituents such as aspiperol A, piperbetol, and piperol B. These components have been reported as strong agents specific for platelet activating factor receptor antagonist [3].

Also, beta-sitosterol and triterpenes have been isolated from Piper betle and showed anti-inflammatory and antiplatelet activities [4]. Majumder et al., [5] have reported the gastro-cytoprotective effect of Piper betle in the lesions induced experiments through an antioxidant mechanism. Intestinal lipase and amylase activity have been stimulated by the Piper betle [6].

Disruption in the defensive and aggressive mucosal factors and antioxidant imbalance could be one of the critical factors for peptic ulcer [7]. Acute and chronic stress, alcohol abuse and chronic use of NSAID are considered as the major factors for peptic ulcer. There are several drugs available to treat peptic ulcers such as antacids, H-2 receptor antagonist, anticholinergics and proton pump inhibitors. However, these drugs would significantly produce several side effects [8]. Therefore, these findings indicate the study of the protective role of Piper betle from natural sources.

Therefore, The present study was aimed to investigate the protective effect of Piper betle against nonsteroidal anti-inflammatory drug (NSAID)-induced gastric ulcer in the male albino rats. Lipid peroxidation, catalase, superoxide dismutase (SOD), lactate dehydrogenase (LDH), reactive oxygen species (ROS) were determined.

Materials and methods

Materials

Healthy male albino rats have obtained from the animal house, Shangai, China, weighing (180-200 g) was selected for the present study. They have kept in polypropylene cages, at temperature 25±0.5°C, relative humidity 60±5%
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and a photoperiod of 12 h/day. All the animals were treated according to internationally accepted ethical procedures.

**Preparation of Piper betle extract**

The leaves of *Piper betle* were chopped into fine pieces. Then, it was made into a paste in ethanol forming slime. Then, it was percolated in ethanol for 10 days. After that, the solvent was filtered with use of nylon mesh and it was repeated twice. It was allowed to evaporate the alcohol portions of extract in a rotary evaporator. The final solution was dried and stored for the further use.

**Experimental groups**

The male albino rats have grouped into 4 groups of six rats each. The experimental groups were designated as follows; Group I, Group II, Group III, and Group IV.

- **Group I**: Normal control-15 consecutive days
- **Group II**: Ulcer control-15 consecutive days
- **Group III**: Treatment (10 mg/kg/day)-15 consecutive days
- **Group IV**: Treatment (20 mg/kg/day)-15 consecutive days

At the end of treatment, the animals were killed, blood and tissues were collected for the analysis.

**Determination of lipid peroxidation**

Lipid peroxidation (LPO) was determined by the kit method. This was based on the spectrophotometric method of Muthuraman et al., [9]. MDA was measured by determining the thiobarbituric acid reactive species. The absorbance of the resultant product has measured at 534 nm (Agilent Technologies, Cary 100 UV-Vis spectrophotometer).

**Determination of LDH activity**

LDH was determined in serum using standard kits according to manufacturer’s instruction. The activity of LDH was expressed IU/L [10].

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**Figure 1.** Determination of lipid peroxidation in the NSAID-induced peptic ulcer model of male albino rats. Rats were administered 10 and 20 mg/kg bwt of *Piper betle* for 15 consecutive days. All the values were expressed mean ± SEM. *P<0.05.

**Figure 2.** Determination of LDH activity in the NSAID-induced peptic ulcer model of male albino rats. Rats were administered 10 and 20 mg/kg bwt of *Piper betle* for 15 consecutive days. All the values were expressed mean ± SEM. *P<0.05.
SOD and catalase enzyme activities were determined by using the kit method, which was based on the method of Muthuraman et al., [11].

Catalase activity was measured and expressed as U/g. Catalase activity was significantly increased in the ulcer induced rats (group II). However, the administration of *Piper betle* significantly increased catalase activity in the male albino rats. Catalase activity was significantly increased 30.3 and 80.9% at 10 and 20 mg/kg bwt of *Piper betle* administration in the male albino rats (Figure 1, P<0.05).

**Results and discussion**

Lipid peroxidation was measured as MDA content and expressed as nmol/g. MDA content was significantly increased in the ulcer induced rats (group II). However, the administration of *Piper betle* significantly reduced lipid peroxidation in the male albino rats. Lipid peroxidation was significantly reduced 17.8 and 35.7% at 10 and 20 mg/kg bwt of *Piper betle* administration in the male albino rats (Figure 1, P<0.05). LDH activity was measured and expressed as U/L. LDH activity was significantly increased in the ulcer induced rats (group II). However, the administration of *Piper betle* significantly reduced LDH activity in the male albino rats. LDH activity was significantly reduced 12.2 and 41.5% at 10 and 20 mg/kg bwt of *Piper betle* administration in the male albino rats (Figure 2, P<0.05).

**Determination of ROS**

Rat stomach cells were cultured in a dish. Cells were treated as mentioned in the method section. After treatment, the cells were treated with DCFH-DA for 30 min at 37°C and 5% CO₂. Cells were viewed for fluorescence under a fluorescence microscope (Olympus, Japan) [12].

**Figure 3.** Determination of catalase activity in the NSAID-induced peptic ulcer model of male albino rats. Rats were administered 10 and 20 mg/kg bwt of *Piper betle* for 15 consecutive days. All the values were expressed mean ± SEM. *P<0.05.

**Figure 4.** Determination of SOD activity in the NSAID-induced peptic ulcer model of male albino rats. Rats were administered 10 and 20 mg/kg bwt of *Piper betle* for 15 consecutive days. All the values were expressed mean ± SEM. *P<0.05.
mg/kg bwt of *Piper betle* administration in the male albino rats (Figure 3, *P*<0.05). SOD activity was measured and expressed as U/mg. SOD activity was significantly reduced in the ulcer induced rats (group II). However, the administration of *Piper betle* significantly increased SOD activity in the male albino rats. SOD activity was significantly increased 23.7 and 42.1% at 10 and 20 mg/kg bwt of *Piper betle* administration in the male albino rats (Figure 4, *P*<0.05).

Cells treated with *Piper betle* as mentioned in the method section. The fluorescent probe DCFH-DA determined intracellular ROS generation. The fluorescence intensity of DCF was decreased in the *Piper betle*-treated cells in a dose-dependent manner (Figures 5, 6). The extract of *Piper betle* showed a significant effect on healing of NSAID-induced peptic ulcers. Mucus layer and hexosaminewere gradually increased in the NSAID-induced peptic ulcer rats following *Piper betle* administration, which indicates the protective effect of *Piper betle*.

SOD and catalase enzymes were reached normal levels the NSAID-induced peptic ulcer rats following *Piper betle* administration. Thus,
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Piper betle could act as a free radical scavenger and started to heal the ulcers. In the biological system, the reduction of thiol group is very essential. The increased lipid peroxidation and altered glutathione levels could increase thiol groups in the biological system [13]. The increased mucin turnover rate serves as a protective layer for epithelial digestion system. 

In other hands, it can be assumed that increased free radical scavenging action of mucin could provide a protective layer for epithelial digestion system [14]. Usually, NSAID induces peptic ulcers through the inhibition of prostaglandin synthase and over production of lipooxygenase activity and leukotrienes level [15]. Formation of peptic ulcers leads to the increased levels of neutrophils and xanthine oxidase activity [16-19]. The free radical scavenging action of Piper betle could indicate the peptic ulcers are healing action through the anti-oxidative mechanism.

Conclusion

In summary, taking all these data together, it can be suggested that therapeutic action of Piper betle on peptic ulcer through free radical scavenging action.

Disclosure of conflict of interest

None.

Figure 6. Determination of ROS level in the NSAID-induced peptic ulcer model of male albino rats. Rats were administered 10 and 20 mg/kg bwt of Piper betle for 15 consecutive days. All the values were expressed mean ± SEM. *P<0.05.

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