ANTIULCER ACTIVITY OF PETROLEUM - ETHER EXTRACT OF VANDA TESSELLATE ROXB

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ABSTRACT

Present study was designed to investigate the antiulcer activity of petroleum ether extract of Vanda tessellata leaves. Wistar rats were used and divided into three groups. Group one to three received tween 80 (negative control), aspirin (Positive control), and 200mg/kg of petroleum ether extract with aspirin respectively. After 12 hour of fasting period, aspirin 200mg/kg orally administered to group two and three. Rats were sacrificed 5 hours after aspirin treatment. Stomach was analyzed for histological changes. Aspirin control shown damaged gastric architecture with dead neutrophils and pus formation of gastric mucosal layer, whereas rats pretreated with petroleum ether extract showed few inflammatory cells presented only in the base of the mucosal layer. Results of this study showed that pretreatment with petroleum ether extract of Vanda tessellata provided moderate significant protection against aspirin induced gastric ulcer. Further studies on different animal ulcer models required to confirm the antiulcer activity of petroleum ether extract of Vanda tessellate.

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INTRODUCTION

Aspirin is belonging to the NSAID and it produces undesirable side effects such as gastrointestinal ulcers, stomach bleeding and tinnitus, especially at higher dose or chronic use. Several drugs widely used to prevent these side effects, which include H\textsubscript{2} receptor antagonist and proton pump inhibitors. Due to problems associated with current treatment, there is the need to seek alternative drug source against gastric ulcers.

\textit{Vanda tessellata} is an orchid widely distributed in India and Indochina. \textit{Vanda tessellata} has been used in the indigenous medicine such as Ayurveda and local traditional medical practices \cite{1}. The leaves in the form of a paste are applied to the body to bring down fever \cite{2}. The roots were used in rheumatism, nervous problems, bronchitis and dyspepsia \cite{3}. Roots were reported to possess antibacterial and antitubercural properties \cite{4}. The steroidal fraction obtained from \textit{Vanda tessellata} has been shown significant anti-inflammatory activity against acute inflammation induced by carrageenan, serotonin and formaldehyde \cite{5}. The methanol extract of this plant root also showed anti-inflammatory activity against carrageenan - induced oedema in rodents \cite{6}. It contains many biologically active principles like alkaloid, flavonoids, glycoside, tannins, \(\beta\)-sitosterol and \(\gamma\)-sitosterol. In our previous study we reported the anxiolytic and membrane stabilization activity of \textit{Vanda tessellata} \cite{7, 8}. The present study designed to evaluate the antiulcer activity of pet – ether extract of \textit{Vanda Tessellata} Roxb, as it was not scientifically proven.

MATERIALS AND METHODS:

This study protocol was approved by Institutional Ethical Commitee, Department of Pharmacology at Dr. V.R.K. Women's Medical College. \textit{V. tessellata} leaves were shade dried and one kg of coarse powder was soaked in 4 litres of petroleum-ether for 3 days at room temperature. The extract was evaporated to dryness by using a rotary vacuum flash evaporator and the yield was 10.6\% w/w. The petroleum ether extract was then subjected to qualitative chemical investigation for the identification of phyto constituents like triterpenoids, saponins, alkaloids, carbohydrates, tannins, flavonoids and glycosides using appropriate reagents. The extracts were treated with dilute hydrochloric acid and filtered. The filtrate is used for the experiment.

Animals

Wistar rats (175-200g) were procured from the institutional animal house. The animals had free access to water, but food was restricted during and 12 hours before experiment. The animals were maintained under strict hygienic conditions and room temperature of 25±1°C, relative humidity 45-55\% and a 12:12h light/dark cycle. All the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the study protocol was approved by the institutional animal ethical committee.

Acute toxicity studies

Acute oral toxicity studies were performed according to Organization for Economic Cooperation and Development (OECD 423) guidelines \cite{9}. Wistar rats of either sex weighing 175-200g were used for this purpose. Tween - 80 1\% v/v was used as vehicle to suspend the petroleum-ether extract. The petroleum-ether extract was administered in a dose of 2g/kg orally to a group of three rats. The animals were continuously observed for changes in autonomic or behavioral responses for 6hrs. The animals were kept under observation for 14 days to detect any mortality.

Aspirin induced ulcers in rats \cite{10}

Wistar rats were divided into three groups and each group consists of six animals. The petroleum ether extract of \textit{Vanda tessellata} (200mg/kg) is administered orally in 0.1\% Tween 80 solution for two consecutive days to group -3. After 12 hour fasting period, aspirin (200 mg/kg, 4 mg/ml dissolved in 0.1\% Tween 80 solution) was administered orally to the group -2 and group – 3. Control group treated only with 0.1\% Tween 80 solution. Five hours later, the rats are sacrificed under ether anaesthesia and their stomach was excised. They were opened along the greater curvature and washed in warm water. Then the part of the stomachs was fixed in buffereal formalin (10\%) solution for histological evaluation. The fixed stomachs were embedded in paraffin wax to produce paraffin wax tissue sections then 5µm sections with H&E evaluated for microscopical examination.

RESULTS

Phytochemical Screening

The percentage yield of petroleum-ether extract of leaves of \textit{Vanda tessellata} was found to be 10.6\% w/w. The chemical tests indicate the presence of flavonoids, sterols, carbohydrates, tannins, phenols, glycosides and alkaloids in the petroleum ether extract.

Acute toxicity studies

There was no significant alteration in autonomic or behavioral responses in the rats treated with petroleum-ether extract of the leaves of \textit{Vanda tessellata}. No mortality was recorded in these animals up to 14 days. Thus the petroleum-ether extract was found to be non-toxic up to dose of 2g/kg body weight.

Histological examination of the gastric tissue:

Histological examination revealed that, Aspirin group shown extensive damage to gastric mucosa layer with edema and leucocytes infiltration of sub mucosal layer (Fig 1). Protection of gastric mucosa was better as seen by moderate disruption to the surface epithelium mucosa with moderate edema and leucocytes infiltration of the sub mucosal layer after pretreatment with 200mg/kg of petroleum ether extract of \textit{Vanda tessellata} (Fig 3).

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DISCUSSION
The present study was designed to evaluate the antiulcer activity of petroleum ether extract of Vanda tessellata. In acute toxicity testing no mortality was observed in mice even in a dose of 2g/kg of petroleum-ether extract of Vanda tessellata, which indicates the safe nature of the extract. Aspirin is a potent nonsteroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases. Gastric ulcer associated with the use of aspirin is a major problem. Many factors such as gastric acid and pepsin secretion, prostaglandin E2 (PGE2) content, [11] NO synthase (NOS), oxidative damage, proinflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF) [12, 13] play important roles in the genesis of gastric mucosal damage, and its subsequent development. [14,15]. The results on histopathological investigation on the gastric mucosa of rats revealed that the pretreatment with petroleum ether extract of Vanda tessellata moderately inhibited aspirin induced haemorrhage, edema, dysplastic changes and ulceration. The exact mechanism of antiulcer activity of petroleum ether extract of Vanda tessellata was not known, but it could be due to presence of phytochemical constituents in the extract such as flavonoids or phenols [16]. This interesting observation indicates that the extract possess antiulcer activity. Absence of ulcerogenic effect and protection against aspirin induced ulceration opens up new possibilities to the use of petroleum ether extract of Vanda tessellata. However, detailed study like isolation of active molecule and confirmation of its antiulcer activity in other ulcer models is required to confirm the above biological action.

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REFERENCES