GASTROPROTECTIVE ROLE OF VITEX NEGUNDO LINN IN ALBINO RATS WITH ASPIRIN INDUCED ULCER

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Abstract: In the present study, the gastroprotective activity of aqueous extract of Vitex negundo (L) against the gastric mucosal damage induced by aspirin was studied in albino rats. Aspirin was administered intraperitoneally at a dose of 80mg/kg body weight to induce ulcer and the resultant elevated levels of lipid peroxide was taken as an index of oxidative stress. The gastroprotective effect of V. negundo (L) was observed at an oral dose of 200mg/kg body weight administered for 18 days before ulcer induction. The effect of V. negundo (L) on the levels of RBC, WBC, Proteins, carbohydrates, lipid peroxidase, super oxide dismutase and glutathione were investigated in ulcer induced rats and the results revealed that V. negundo (L) has a pivotal role in treating ulcer.

Key words: Vitex negundo (L), Gastroprotection, Aspirin, Ulcer

INTRODUCTION

Peptic ulcer is one of the common diseases in human population and its incidence generally varies from 3-10% [1]. It can be attributed to either Helicobacter pylori or non-steroidal anti-inflammatory drugs (NSAIDs) induced mucosal damage [2] besides an imbalance between aggressive and defensive factors [3,4]. Aspirin is a common NSAID usually used to treat swelling, inflammation, to relieve pain and fever. But it also has lot of side effects among which gastrointestinal (GI) toxicity deserves main concern as it is associated with significant morbidity and mortality. The relative risks for the occurrence of gastric and duodenal ulcers in chronic aspirin users were estimated to be 4.7 and 1.2 % respectively and the relative risks for GI bleeding was 3.3 % for aspirin users [5].

The efficacy of aspirin in the prophylaxis of various ocular diseases depends upon its inhibition of prostaglandin synthesis through its irreversible acetylation of cyclooxygenase. But prostaglandins are important cytoprotective agents in the GI tract because they increase mucosal blood flow [6]. Inhibition of prostaglandin synthesis by aspirin causes damage to the cell membrane of mucosal, parietal and endothelial cells [7]. The present work focuses on the antiulcerogenic effects of V. negundo (L), as the experimental evidences for its antiulcer activity are not abundant.

V. negundo (L) is a deciduous shrub belonging to the family Verbenaceae comprising 75 genera and nearly 2500 species, chiefly occurring in Pakistan, India and Ceylon [8,9]. Though almost all plant parts are used, the extract from leaves and roots is the most important in the field of medicine as drugs. The decoction of leaves is considered to be a tonic, vermifuge and is given along with long pepper in catarrhal fever [10]. Water extract of matured fresh leaves exhibited anti-inflammatory, analgesic and antihistamine properties [11]. Phytochemical screening studies of V. negundo (L) revealed the
presence of volatile oil [12], triterpenes [13],
diterpenes [14], sesquiterpenes [15], lignin [16],
flavonoids [17], flavone glycosides [18] and stilbene
derivative [19]. V. negundo (L) is an aromatic shrub
with a variety of medicinal uses and this study
explores the gastro protective action and antiulcer
activity of this plant.

**MATERIALS AND METHODS**

**Drug preparation:** Dried leaves of V. negundo (L)
were taken and water extract was prepared by
continuous extraction method with the help of Soxhlet
apparatus. After vacuum evaporation the crude
extract was suspended in water.

**Animals:** The study was conducted on albino Wistar
rats weighing 150-200 g and maintained under
standard conditions (room temperature of 24 to 27
°C and humidity of 60-65%) with 12 hrs of light and
dark cycle. The food in the form of dry pellets
(Saidurga Feeds, Bangalore) and water were
available ad libitum. The experiments were
conducted according to the ethical guidelines of
CPCSEA (Committee for the Purpose of Control and
Supervision of Experiments on Animals) after
obtaining necessary clearance from the committee
(Approval No: 790/03/ac/CPCSEA).

**Experimental model:** Albino rats were divided into
groups of five each comprising six rats.
Group I (control) received only normal food and
water. Group II received Aspirin (80mg/kg body
weight) on 19th, 22nd and 25th days of the experiment.
Group III received leaf extract of V. negundo (L)
(100mg/kg/day for 28 days + Aspirin(80 mg/kg)) on
19th, 22nd and 25th days. Group IV received leaf
extract of V. negundo (L) (200mg/kg/day for 28
days + Aspirin(80 mg/kg)) on 19th, 22nd and 25th days.
Group V received only V. negundo (L) (200 mg/
Kg) for 28 days. On the 29th day the animals were
sacrificed. The blood was collected through heart
puncture and serum was separated for analyzing
various biochemical parameters.

**Biochemical investigations:** Total carbohydrates
[20], total protein [21] and the levels of glutathione
(GSH) [22] and super oxide dismutase (SOD) [23]
were estimated by appropriate methods. The levels
of serum glutamate oxaloacetate transaminase
(SGOT) and serum glutamate pyruvate transaminase
(SGPT) were determined by the method of King [24].

**Haematological investigation:** The levels of total
RBC, WBC and Hb were estimated [25].

**Histological studies:** For histological study, the
tissues (liver, kidney and stomach) were fixed in
Bouin’s fluid. The classical paraffin sectioning and
haematoxylin eosin staining techniques were used for
histological studies [26]. The histochemical sections
were evaluated by light microscopy.

**Statistical evaluation:** The results obtained were
expressed as mean ± SD and were analyzed by the
application of one-way analysis of variance
(ANOVA) [27].

**RESULTS AND DISCUSSION**

The levels of LPO, SOD and GSH were estimated
and the results are given in table 1. It was found that
aspirin treated rats (Group II) showed a remarkable
elevation in the level of LPO, and significant reduction
in the level of SOD and GSH. However, a marked
increase in SOD and GSH levels and decrease in
the level of LPO were found in rats pre-treated with
200mg/Kg body weight of V. negundo (L) (Group
IV).

Group IV animals pretreated with V. negundo (L)
showed a gradual increase in the level of carbohy-
drates and decrease in protein when compared to
group II rats (Table 3). Rats with aspirin induced
ulcer (Group II) showed a significant reduction in
RBC, WBC and Hb levels as compared to disease
control group, while a significant elevation in total
RBC, WBC and Hb was found in the aspirin induced
animals pre-treated with an aqueous extract of V.
negundo (L) at 200mg/Kg body weight for 18 days before ulcer induction (Table 2).

The effects of V. negundo (L) on different serum
marker enzymes are represented in table 4. The levels
of amylase, alanine aminotransferase (ALT) and
aspartate aminotransferase (AST) were markedly
elevated in aspirin induced rats (Group II) as
compared to control indicating gastric damage
followed by liver damage. However, a significant
reduction in the levels of amylase, ALT, AST were
found in aspirin induced rats pre-treated with 200mg/
Kg body weight of V. negundo (L) (Group IV).

In the microscopic observation, normal arrangement
of gastric cells was found in control rats (Fig 1).
Fig. 1: Histological examination of stomach of control rats showed normal arrangement of mucosal layer, gastric cells and no hemorrhage. x 200

Fig. 2: Histologically, Aspirin induced Group II animals showed the degeneration, hemorrhage and edematous appearance of the gastric mucosal tissue. Aspirin treated group showed a significant decrease of mucosal thickness compared to control group. x 200

Fig. 3: The Group IV rats pretreated with aqueous leaf extract of *V. negundo* (L) significantly inhibited the gastric lesions formation and sub-mucosal edema compared to Group II animals induced by aspirin. After pretreatment with aqueous leaf extract of *V. negundo* (L), Aspirin showed a significant mucosal thickness as compared to aspirin treated group. x 200
### Table 1: Effect of *V. negundo* (L) on levels of SOD, GSH and LPO in experimental animals. *compared between normal and disease control. **compared between disease control and drug treated (200mg/kg bw)*

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>SOD (U/g/min)</th>
<th>Glutathione (U/g/min)</th>
<th>LPO (U/g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>68.5±1.870*</td>
<td>6.7±0.1581*</td>
<td>46±1.581*</td>
</tr>
<tr>
<td>Group II</td>
<td>14.0±0.811*</td>
<td>1.98±0.1923*</td>
<td>95.8±1.923*</td>
</tr>
<tr>
<td>Group III</td>
<td>41.1±10431</td>
<td>4.61±0.1746</td>
<td>64±2.23</td>
</tr>
<tr>
<td>Group IV</td>
<td>61.2±1.923**</td>
<td>6.26±0.2073**</td>
<td>49.1±1.764**</td>
</tr>
<tr>
<td>Group V</td>
<td>66.1±1.596</td>
<td>6.48±0.0836</td>
<td>48±1.431</td>
</tr>
</tbody>
</table>

### Table 2: Effect of *V. negundo* (L) on levels of RBC, WBC and haemoglobin in experimental animals. *compared between normal and disease control. **compared between disease control and drug treated (200mg/kg bw)*

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>RBC (millions of cells/cu mm)</th>
<th>WBC (Thousands of cells/cu mm)</th>
<th>Hb (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>4.068±0.0519*</td>
<td>5.206±0.0697*</td>
<td>8.4±0.2828*</td>
</tr>
<tr>
<td>Group II</td>
<td>1.222±0.014*</td>
<td>1.294±0.0149*</td>
<td>2.4±0.1414*</td>
</tr>
<tr>
<td>Group III</td>
<td>2.03±0.810</td>
<td>3.84±0.0282</td>
<td>6.10±0.1083</td>
</tr>
<tr>
<td>Group IV</td>
<td>3.956±0.571**</td>
<td>4.902±0.0581**</td>
<td>8±0.0707**</td>
</tr>
<tr>
<td>Group V</td>
<td>3.94±0.03</td>
<td>5.072±0.710</td>
<td>4±0.028</td>
</tr>
</tbody>
</table>

### Table 3: Effect of *V. negundo* (L) on levels of proteins and carbohydrates in experimental animals. *compared between normal and disease control. **compared between disease control and drug treated (200mg/kg bw)*

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Protein (g/dl)</th>
<th>Carbohydrate (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>8.85±0.0707*</td>
<td>0.62±0.014*</td>
</tr>
<tr>
<td>Group II</td>
<td>2.15±0.1*</td>
<td>2.75±0.0707*</td>
</tr>
<tr>
<td>Group III</td>
<td>5.14±0.086</td>
<td>1.98±0.086</td>
</tr>
<tr>
<td>Group IV</td>
<td>8.25±0.0707**</td>
<td>0.77±0.0927**</td>
</tr>
<tr>
<td>Group V</td>
<td>8.73±0.923</td>
<td>0.778±0.0213</td>
</tr>
</tbody>
</table>

### Table 4: Effect of *V. negundo* (L) on levels of SGOT and SGPT in experimental animals. *compared between normal and disease control. **compared between disease control and drug treated (200mg/kg bw)*

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>49±1.581*</td>
<td>61.33±2.160*</td>
</tr>
<tr>
<td>Group II</td>
<td>170.8±1.924*</td>
<td>190±1.581*</td>
</tr>
<tr>
<td>Group III</td>
<td>88±1.162</td>
<td>81.2±1.923</td>
</tr>
<tr>
<td>Group IV</td>
<td>64.2±1.483*</td>
<td>68.4±2.073*</td>
</tr>
<tr>
<td>Group V</td>
<td>47.8±1.047</td>
<td>62.1±1.746</td>
</tr>
</tbody>
</table>
Aspirin induced rats showed an ulcer crater indicating gastric lesion with, damaged mucosal epithelium and acute inflammation in the stomach (Fig. 2). In comparison maintenance of muscularis mucosa and a reduced size of ulcer crater was observed in V. negundo (L) pre-treated rats (Fig. 3). The histological studies of V. negundo (L) alone treated rats showed normal arrangement of gastric mucosa, kidney and liver cells with no pathological changes.

Mucus secretion is a crucial factor in the protection of gastric mucosa from the gastric lesions and has been regarded as an important defensive factor in the gastric mucous barrier [28]. Prostaglandins are important cyclo protective agents in the gastro intestinal track because they increase mucous secretion, bicarbonate secretion and mucosal blood flow. Hydrophobic surfactant like phospholipid secretion in the gastric epithelial cells is also stimulated by the prostaglandin [29]. They also stabilize mucosal mast cells, lysosomal membranes and inhibit free radical production. Aspirin is a potent inhibitor of prostaglandin synthesis through its irreversible acetylation of cyclooxygenase. This inhibition is one of the main reasons for mucosal injury in the stomach and duodenum. Aspirin also breaks the gastric mucosal barrier by non prostaglandin dependent mechanisms leading to a reduction in mucosal potential difference and back diffusion of hydrogen ions [6]. In the present study the increase in the level of carbohydrates in aspirin induced rats pretreated with Vitex negundo (L) is a direct reflection of mucin activity and decrease in protein content in the gastric juice also signifies the decrease in leakage from mucosal cells indicating mucosal resistance.

Antioxidant enzyme like SOD and non enzymatic antioxidant like GSH are the first line of defense against lipid peroxidation [30]. They are highly specific in their catalytic mode of action and decrease the gastric mucosal damage against free radicals. The results of the present investigation substantiated this view as the levels of SOD and GSH were found to be higher in aspirin induced rats pre-treated with V. negundo (L) than in rats with aspirin induced ulcer (Group II) confirming its gastroprotective activity. V. negundo (L) offers gastric protection against aspirin induced ulcer by significantly blocking lipid peroxidation which is proved by the reduced levels of lipid peroxide in Group IV animals. The above results are in accordance with previous research experiments and confirm the anti-oxidant activity of V. negundo (L) [31].

Ulcer healing is a complex process involving a combination of wound retraction and re-epithelialization. In histological study, V. negundo (L) pretreatment showed not only the maintenance but also the regeneration of gastric mucosa in the damaged region. The elevated levels of AST (aspartate transaminase) and ALT (alanine transaminase) in Group II rats may be due to leakage of the enzymes from damaged hepatic cells caused by aspirin, which not only affects gastric mucosa but also damages liver cells. The pre-treatment with V. negundo (L) in group IV rats significantly decreased the levels of AST and ALT, and this is due to the hepatoprotective activity of V. negundo (L) as also evidenced by Avadhoot and Rana [32].

Overall this study proves the hepatoprotective and gastroprotective activities of V. negundo (L) in aspirin induced ulcerated rats. Preliminary phytochemical studies of V. negundo (L) have revealed the presence of flavonoids [17], which shows anti-ulcerogenic and gastroprotective activities [33]. This indicates that the gastroprotection by Vnegundo, as observed in the present investigation, may be due to the presence of flavonoids.

REFERENCES

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