EVALUATION OF ULCER-PROTECTIVE AND ANTIMICROBIAL ACTIVITY OF SYZYGIUM CUMINI (LINN.) SKEELS LEAVES

Sushil Bhargava, Paridhi Bhargava and U. K. Jain*

Dept. of Pharmacognosy and Herbal Drug Research, Bhopal Institute Technology & Sciences-Pharmacy Bhopal India

Summary

This study examines the Ulcer-protective and antimicrobial activity of aqueous extracts of Syzygium cumini (Linn.) Skeels leaves. For anti-ulcerogenic activity, Gastric ulcer was induced in experimental rats by administration of Hard liquor (48% ethanol) available in the market (1ml/150gm body weight) and by administration Aspirin (200mg/kg, p.o.). Extract (100µg/Disc) was also evaluated for their antimicrobial activity by Disc Diffusion method. Aqueous extract of Syzygium cumuni (Linn.) skeels (200 and 400mg/kg p.o.) leaves showed significant ulcer protective activity (p<0.001). Antimicrobial activity on selective strain showed in dose dependent manner in comparison to standards. For anti-ulcer activity Lanceprazole was taken as standard and antimicrobial activity Ofloxacin and Miconazole were used as standard.

Keywords: Jamun, ulcer, folk medicine, antimicrobial

*Corresponding Author
Prof (Dr.) U. K. Jain
Head, Dept. of Pharmacognosy and Herbal Drug Research, Principal,
Bhopal Institute Technology & Sciences-Pharmacy Bhopal M.P. India
bhargavasushil7@gmail.com or umeshkjain65@gmail.com
Introduction

Ayurveda and other Indian literature mention the use of plants in the treatment of various human ailments [1]. Number of herbal drugs stated in the Ayurvedic system and Indian folk medicine, in which, *Syzygium cumini* linn. Skeel (SC) (family *Myrtaceae*) is being widely used to treat diabetes by the traditional practitioners over many centuries [2]. *Syzygium cumini* is commonly called as Jamun, Black plum or Indian Black berry. It is a large tree found in all forest over the greater part of India from the sub-Himalaya to extreme south in forest up to 1,800 m usually along river bank and most localities, also cultivated as shade tree and for its edible fruits along road sides [3, 4]. It is also found in Thailand and Philippines. The fruits of SC are oval to elliptical, 1.5–3.5 cm long, dark purple or nearly black, luscious, fleshy and edible [5]. It is widely used in the treatment of Diabetes mellitus, Dysentery, Diarrhoea, and Ulcer [6]. The plant has been reported as Anti-diabetic [7], Anti-oxidant [8], Anti-inflammatory [9], Anti-bacterial [10], and Anti-convulsant [11]. Plant contains phytochemicals like Dihydromyricetin, Ellagic acid, Quercetin, Triterpenoids, Gallic acid, Mallic acid, Ferulic acid, Sitisterol, Glycine, Leucine, Betulinic acid, Tannins, Guaiacol etc.[3]. Thus an effort has been made to establish the anti-ulcer as well as antimicrobial activity of aqueous extract of SC (AESC).

Methodology

Plant Material
Fresh leaves of *Syzygium cumini* Linn. were collected from forest of Sanjivani Udyana (Herbal Garden) Bhopal (M.P.), India in July 2008 and identified by Professor (Dr.) U. H. Khan [Retd. Botanist and Taxonomist, Govt. Agriculture College Jabalpur (M.P.)] and a voucher specimen (BITSP/007/2007) was deposited for reference to Department of Pharmacognosy, BITS-Pharmacy.

Preparation of Extract
Shade-dried leaves powdered and soxhlet extracted with distilled water. The extract was concentrated by rotary vacuum evaporator. The dried extract was stored in air tight container in refrigerator below 10°C. and weighed quantity was suspended in 2% Tragacanth solution for the experiment.
Experimental Animals
Wistar albino rats (150±20 g) and Albino mice weights about 25±5 g were used for the studies of the crude extracts. Institution Animal Ethics Committee has approved the project (1918/cc/05/CPCSEA). The animals were kept in departmental animal house in well cross ventilated room at 27±2°C, relative humidity 44–56% and light and dark cycles of 10 and 14 h respectively for 1 week before and during the experiments. Animals were provided with standard diet (Lipton, India) and the food was withdrawn 18–24 h before the start of the experiment and water ad libitum.

Determination of acute toxicity (LD50)
The acute toxicity for AESC was determined in wistar mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted for toxicity studies.[12]

Anti ulcer activity
Gastric ulceration was induced in 24 hrs. fasted rats by the administration of necrotizing agent like hard liquor (42.8% ethanol at 10ml/kg. p.o.) and Aspirin (200mg/kg, p.o.) to group of 6 animals each were pre-treated with AESC (200mg/kg, p.o. and 400mg/kg p.o.),1 hr. before the ulcerogenic procedures. [13]
The animals were sacrificed after 6 hr. after administration of ulcerogenic drugs and 1 hr. after the administration of necrotizing agent by an overdose of hard liquor. The stomachs were removed and opened along with the greater curvature of stomach; the ulcer index was evaluated according to severity and score. The scores were: 0 = no ulcer, 1 = superficial ulcer, 2 = deep ulcer and 3 = perforation. Ulcer area was assessed by using 3M® scaled surgical transpore tapes, which was fixed on a light and transparent sheet. Each cell on the tape was 1 mm² in area, so the number of cells was counted and the ulcer area was measured. Ulcer index was measured by the following formula using the factor of ulcer area instead of ulcer prevalence. Lansoprazole (8mg/kg, p.o.) was used as an antiulcer standard [14].

\[
UI = UN + US + UA \times 10^{-1}
\]
In which UI = ulcer index,
UN = ulcer number, US = ulcer score, and UA = ulcer surface area of stomach.
Antimicrobial Assay:

Seeded broth: The strains of microorganisms inoculated in conical flask containing 100 ml of nutrient broth. These conical flask were incubated at 370°C for 24 hrs and was referred to as seeded broth.

Culture Media: The nutrient agar media (Hi- media, Mumbai) was prepared by dissolving 28 gms of nutrient agar in 100 ml of distilled water. The nutrient broth media (Hi- media, Mumbai) was prepared by dissolving 13 gms of nutrient broth in 100 ml of distilled water. The media was sterilized by autoclaving at 15lb/sq.inch pressure at 121°C for 20 minutes. The paper disc diffusion method for antibiotic susceptibility testing (Malcolm et al., 1969) was used. Strain of gram-positive bacteria S. Aureus (NCIM 2079), B. Subtilis (NICM 2063), and the gram-negative bacteria K. pneumoniae (NICM 2597), and E. coli (NICM 2931) were used in this study. Two yeast strains C. Krusei (NCIM364), and C. Albicans (NCIM347) were also used in this study. Paper disc of 6 mm diameter were prepared using aqueous extract (100µg/disc) of Syzygium cumini Linn and ofloxacin (5µg/disc) and Micanozole (40µg/disc) as standard were used and discs were dried at 37°C before use. The bacterial broth suspension (seeded broth) was streaked evenly on the surface of a medium with a cotton swab. Subsequently the paper discs were placed on the surface of agar with flame forceps and gently pressed down to ensure contact. Plates were incubated at 37°C overnight. After 4 hrs. of incubation, the inhibition zone diameters (including the 6 mm disc) were measured with calipers. A reading of more than 6 mm indicated growth inhibition

Determination of Zone of Inhibition: The disc diffusion method of drug potency is based on the measurement of the diameter of zones of microbial growth inhibition surrounding discs containing various concentration of test compound, which are placed on the surface of a solid nutrient previously inoculated with culture of suitable microorganisms. Inhibition produced by the test drug was compared with that produced by known concentration of reference standard drug. [15]
Results

**Acute Toxicity Study**
AESC showed no mortality at 2000 mg/kg, so 1/10\textsuperscript{th} and 1/5\textsuperscript{th} of that were selected (200mg/kg and 400mg/kg dose) for all in-vivo experiments as sub-maximal and maximal dose.

**Anti-ulcer (ulcer-preventive) activity study**
Pretreatment of rats with AESC rendered a dose-dependent protection from gastric mucosal damages induced by hard liquor and Aspirin (necrotizing agent). Effect of AESC on gastric ulcers induced by Hard liquor in rats with comparison to the ulcer control animals, AESC produce 32.17\% and 61.09\% ulcer inhibition (%) respectively at 200 and 400 mg/kg doses (Table 1). Effect of AESC on gastric ulcers induced by Aspirin in rat shows 23.01\% and 70.33\% ulcer inhibition (%) respectively at 200 and 400 mg/kg doses (Table 2).

**Table 1. Effect of AESC on gastric ulcers induced by Hard liquor in rats.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg/p.o</th>
<th>Mean Ulcer index</th>
<th>Ulcer Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(2% Tragacath)</td>
<td>5ml/kg</td>
<td>0.1 ± 0.100</td>
<td>------</td>
</tr>
<tr>
<td>Control(+ve)</td>
<td>1ml/150gm</td>
<td>4.91 ± 0.08</td>
<td>------</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>8mg/kg</td>
<td>0.5 ± 0.22</td>
<td>89.81</td>
</tr>
<tr>
<td>AESC</td>
<td>200 mg/kg</td>
<td>3.33± 0.57</td>
<td>32.17</td>
</tr>
<tr>
<td>AESC</td>
<td>400 mg/kg</td>
<td>1.91 ± 0.35</td>
<td>61.09</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6); * p<0.001
Table 2. Effect of AESC on gastric ulcers induced by Aspirin in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg/p.o</th>
<th>Mean Ulcer index</th>
<th>Ulcer Inhibition(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(2% Tragacath)</td>
<td>5ml/kg</td>
<td>0.1 ±0.10</td>
<td>-</td>
</tr>
<tr>
<td>Control(+ ve)</td>
<td>200mg/kg</td>
<td>3.91 ± 0.41</td>
<td>-</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>8mg/kg</td>
<td>0.75 ± 0.25</td>
<td>80.8</td>
</tr>
<tr>
<td>AESC</td>
<td>200 mg/kg</td>
<td>3.01 ± 0.44</td>
<td>23.01</td>
</tr>
<tr>
<td>AESC</td>
<td>400 mg/kg</td>
<td>1.16 ± 0.46</td>
<td>70.33</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6); * p<0.001

Antimicrobial activity:
The results of the antimicrobial activity of the AESC (100µg/Disc), assayed in-vitro by disc diffusion method, are shown in Table 3. The AESC (100µg/Disc) displayed the highest level of activity against Candida krusei. Overall the AESC is significantly active against gram positive bacterial strain like S. aureus and B. subtilis and highly active against C. albicans (NCIM347) C. Krusei (NCIM364) whereas less effective against gram negative bacteria K. pneumoniae and E. coli, in comparison to standard.

Table 3 Effect of AESC on zone of inhibition of microorganism

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Microorganisms</th>
<th>ZONE OF INHIBITION (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AESC (100µg/Disc)</td>
</tr>
<tr>
<td>1</td>
<td>E.Coli (NCIM2931)</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>B.Subtilis (NCIM2063)</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>S.Aurus(NCIM2079)</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>K. Pneumoniae(NCIM295)</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>C. Albicans (NCIM347)</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>C. Krusei(NCIM364)</td>
<td>23</td>
</tr>
</tbody>
</table>
Discussion

Peptic ulcer is defined as disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation. Despite the constant attack on the gastroduodenal mucosa by a host of noxious agents (acid, pepsin, bile acids, pancreatic enzymes, drugs, and bacteria), integrity is maintained by an intricate system that provides mucosal defense and repair. This intricate biologic system consist of mucus-bicarbonate layer, surface epithelial cells and a rich submucosal micro-circulatory bed which provides bicarbonate ions to neutralize the acid generated by parietal cell secretion of hydrochloric acid. Moreover, this microcirculatory bed provides an adequate supply of micronutrients and oxygen while removing toxic metabolic by products. Aspirin induced ulcers cause mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H+ ions and thus leading to breaking up of mucosal barrier. [16]

There are reports that alcohol increases the secretion of protein into the gastric juice. Similarly in ethanol induced ulcer GSH level is reduced in gastric mucosa. The gastric GPX level is also reduced. Ethanol induced ulcers are caused by different factors including decreased mucosal blood flow, damage to capillary endothelium and release of arachidonate metabolites specifically LTC4/D4, PAF and histamine [17].

The present study indicated that aqueous extract Syzygium cumini showed ulcer protective effects against ethanol and aspirin-induced gastric ulcers in rats. Syzygium cumini is known to be very rich in tannins and other phenolic constituents. Tannins with its protein precipitating and vaso-constricting effects could be advantageous in preventing ulcer development. Tannins also being an astringent may have precipitated, microproteins on the site of ulcer there by forming and protective film over the lining to prevent absorption of toxic substances and resist the attachment of proteolytic enzymes [18]. Aqueous extract Syzygium cumini showed significant protection against aspirin induced ulcer. It could decrease the acid secretion and increased the mucous secretion which could be capable of preventing back diffusion of H+ ions.
The antimicrobial activity of the aqueous extract *Syzygium cumini* leaves may be due to tannins and other phenolic constituents. The results obtained in this study suggest a potential application of *Syzygium cumini* for treatment of skin wounds and further investigations should be conducted in order to explore this application.

**Conclusion**

Present study reveals that *Syzygium cumini* Linn. Skeel (Jamun) has significant ulcer protective activity and significant antimicrobial activity on selected strain. This study supports to identify exact mechanism and key phytochemical responsible to antimicrobial activity of *Syzygium cumini* Linn. Skeel. Further work on other parameters like gastric mucosal expression and release of tumor necrosis factor-α, interleukin-1β, vascular endothelium growth factor, platelet endothelial cell adhesion molecule-1 which is affected in gastric ulcer may throw more light in understanding the mechanism of ulcer protection by *Syzygium cumini* Linn. Skeel.

**References**


12. Prema Veerarghavan, expert consultant, CPCSEA (Guideline No. 420 of CPCSEA).


