SCREENING OF METHANOLIC EXTRACT OF STERCULIA SCAPHIGERA WALL SEEDS FOR ULCERPROTECTIVE & ANTIOXIDANT ACTIVITY

Sunita C. Ogale*1, Sanjay B. Kasture1, Veena S. Kasture2, Roshan Tiwari3, Zaid Temrikar4

1Department of Pharmacology, VIVA Institute of Pharmacy, Virar (E), Palghar, India
1Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Dist. Ahmednagar, India

ABSTRACT

In this study the antiulcer and antioxidant activity of methanolic extract & methanolic fraction of Sterculia scaphigera wall seeds (MESS & MFSS) was investigated. The methods used to induce ulcer were by physical method i.e. by pyloric- ligation (4 hr.) and by chemical method i.e. administering ethanol orally (40%,2 ml/100gm, for 15 days, twice daily). Pretreatment of rats with the methanolic extract (50 mg/kg body weight, i.p. and p.o.) and methanolic fraction (25 and 50 mg/kg body weight, p.o.) of Sterculia scaphigera markedly reduced pyloric- ligation and ethanol - induced rise in volume of gastric acid secretion, total gastric acidity, pH of gastric content and ulcer index which was supported by the limited extent of histological damage.

KEY WORDS: Sterculia scaphigera, Antioxidant, Antiulcer, Ethanol sunita ogale.

INTRODUCTION

Sterculia scaphigera Wall is a tropical herb of the Sterculiaceae family, mainly distributed in Vietnam, Thailand, Malaysia, Indonesia, and South China[1] (Wang et al., 2003). In Chinese system of medicine, this plant is commonly used for the treatment of phlegm and relieving sore throat, and to relieve constipation[2] (Xiao P. G., 2002). In India, seeds of this plant are available in Herbal Pharmacies for treatment of constipation and hyperacidity. The water
infusion of this herb can promote peristalsis of the intestines\cite{3}. However, there is no systematic scientific study indicating these effects of *Sterculia scaphigera*. The seeds contain alkaloids, sterculin, bassorin, tannins and some astringents\cite{9} (ENaturalHealthCenter.com (e2121.com)).

Since liver is the major drug metabolizing and detoxifying organ in the body, it is most vulnerable to damage. Ethanol consumption is considered to be a risk factor in the development of liver damage. Ethanol when administered chronically is known to potentiate hepatotoxicity of carbon-tetrachloride (CCl$_4$). Alcoholic liver disease (ALD) is the common consequence of prolong and heavy alcohol intake. The fatal changes in the liver include fatty liver, hepatitis and hepatic cirrhosis\cite{4} (Seitz et al.,2005). Reactive oxygen species (ROS) and other free radicals believe to be the key mechanism of ALD\cite{5} (Lindros K.O.,1995). It is well established that CCl$_4$ is metabolized in the liver to highly reactive trichloromethyl radical which initiate free radical-mediated lipid peroxidation of the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane leading to accumulation of lipid-derived oxidants causing liver injury\cite{6,7} (Recknagel R.O.,1967; Recknagel et al.,1989). It also induces hydropic degeneration, centrilobular necrosis, fatty changes, cirrhosis and hepatoma\cite{8} (Smuckler et al.,1962). In the absence of reliable liver protective drugs, herbs may play role in relieving liver disorders. Many plants having antioxidant activity also demonstrate hepatoprotective activities\cite{9} (De et al.,1996). The preliminary studies indicated that the methanolic extract and its methanol soluble fraction possess antioxidant activity in the DPPH assay. Therefore the present study aims to investigate the protective effect of methanolic extract and methanolic fraction of *Sterculia scaphigera* on rat liver damage induced by ethanol-CCl$_4$.

**MATERIALS AND METHODS**

**Preparation of extract**

The seeds of *Sterculia scaphigera* were purchased from Herbal Pharmacy at Nashik and authenticated at the Agharkar Research Institute, Pune. The seeds (0.5 kg) were crushed to a coarse powder and were defatted with petroleum ether (60-80°C) and then extracted with methanol using cold extraction methods with occasional stirring for seven day. The extract was dried in air (yield - 32g). The extract was stored in refrigerator and reconstituted in distilled water just before use. The methanolic extract (8 g) was further fractionated with n-hexane, ethyl aceate and methanol. The yield of methanolic fraction was approximately 1g.
Drugs and Chemicals

1,1-diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma-Aldrich, Mumbai. Standard drug Ranitidine (GSK, India), Alcohol (Qualigens Fine Chemicals, Mumbai), were used. All other reagents used were of analytical grade. All drug solutions were freshly prepared before each experiment. Sterculia scaphigera extract & fraction were dissolved in distilled water and administered orally.

Animals

Wistar Albino rats (150-200g) were obtained from Serum Institute, Pune. Animals were housed five per cage under standard laboratory conditions of temperature (25 ± 2°C) with food and water and relative humidity of 45-55% and 12/12 light/dark cycle. The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and the Institutional Animal Ethical Committee (IAEC) approved protocol of this study.


Acute toxicity: Rat were treated orally with 2 g/kg of MESS & MFSS using up and down method (OECD guidelines) and mortality, if any, was observed for 24 hour.

Antioxidant activity: The antioxidant activity or the inhibition of the generation of free radicals is important in the protection against ethanol-CCl₄ induced hepatopathy[12] (Castro et al.,1974) The ability of test agent to scavenge free radical was determined by using 1,1-diphenyl, 2-picrylhydrazyl (DPPH) assay as described by Hatano[13](Hatano.,1988) . The known quantity of extract and its fraction were dissolved in methanol and the solutions were serially diluted. The dilutions of the methanolic extract and its methanolic fraction were added to solution of DPPH. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517nm using spectrophotometer. L-ascorbic acid was used as reference standard. The % inhibition was calculated from the following equation:

% inhibition = [(absorbance of control - absorbance of test sample)/absorbance of control] x 100%.
The antioxidant activity of each sample was expressed in terms of IC₅₀ (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the calibration curve.

**Antiulcer activity**

**Pyloric ligation induced ulcer**

Rats in a group of five were fasted 48 h prior to receiving dose of the vehicle, MESS (50mg/kg, orally & i.p.), MFSS (25 & 50 mg/kg, orally) and ranitidine (10 mg/kg, orally & i.p.). Pyloric ligation was done by ligating the pyloric end of the stomach of rats 1 h after drug administration[14] (Shay et al., 1945). Rats were allowed to recover and stabilize in individual cage and were deprived of water during post-operative period. After 4 h of surgery, rats were sacrificed and gastric juice was collected and subjected to analysis. Gastric contents were analyzed for total acidity by titrating against 0.01N NaOH using phenolphthalein as indicator. The number of ulcers was noted and the severity recorded as described by Kulkarni, 1999[15].

**Alcohol induced ulcers in rats**

Rats in groups of five each were treated orally with vehicle, ethanol (40%, 2ml/100gm, p.o.), ranitidine (10 mg/kg, orally) & MESS (50mg/kg, orally & i.p.), MFSS (25 & 50 mg/kg, orally) for 15 days twice a day. On day 16th, the animals were killed by an overdose of ether. The abdomen was immediately opened to remove the stomach and ulcer index was calculated as described above.

**Histopathological study** : Immediately after the sacrifice of the anesthetized animals, their stomach tissues were removed and immediately fixed in 10% buffered formalin. These tissues were processed and embedded in paraffin wax after being dehydrated in alcohol and subsequently cleared with xylene. Five-micrometer thick serial histological sections were obtained from the paraffin blocks and stained with hematoxylin and eosin. The sections were examined under light microscope and photomicrographs were taken[16]( Luna, 1996).

**Statistical analysis**: All values are mean ± S.E.M. obtained from six animals. For statistical analysis, One-Way ANOVA with Dunnet’s Test was used to compare the groups. In all the cases a difference was considered significant when p was <0.05.
RESULTS

Phytochemical screening
Phytochemical screening of methanolic extract and methanolic fraction of *Sterculia scaphigera* showed that it contained tannins, terpenoids, alkaloids, amino acids, polysaccharides and flavonoids.

Acute toxicity
There was no mortality in rats treated with MESS & MFSS upto 2 g/kg, p.o.

Antioxidant activity
The IC$_{50}$ of methanolic extract, its methanol soluble fraction and L-ascorbic acid were found to be 200 μg/ml, 98 μg/ml, and 7 μg/ml respectively.

Antiulcer activity
Pyloric ligation induced ulcer
In pyloric ligation model, MESS (50 mg/kg, orally & i.p.), MFSS (25 & 50 mg/kg, orally) produced significant (p<0.05) reduction in ulcer index and also significantly reduced the gastric volume, total acidity, and increased the pH of the gastric fluid, proving its antisecretory activity when compared with control group. As compared to control. But there was no significant decrease in volume. Ranitidine (10 mg/kg, orally & i.p.) also significantly (P < 0.01) reduced ulcer index, gastric volume, total acidity, and increased the pH of the gastric fluid, of pyloric-ligation induced gastric ulcers. Observations are given in Table 1.

Alcohol induced ulcers in rats
Administration of ethanol produced haemorrhagic gastric lesions in the gastric mucosa of the control group. Administration of methanolic extract of *Sterculia scaphigera* and its methanolic fraction reduced these lesions as evidenced by a significant (P < 0.01) reduction in the ulcer index when compared with the group receiving ethanol alone. Ranitidine also significantly (P < 0.01) reduced ulcer index of ethanol-induced gastric ulcers. Observations are given in Table 2.

Group I --- Normal saline (Vehical control)
Group II ---Control group 40% ethanol
Group III ---Ranitidine (10 mg/kg, p.o.) + 40% ethanol
Group IV---MeOH extract of *Sterculia scaphigera* (50mg/kg, i.p.) + 40% ethanol
Group V---MeOH extract of *Sterculia scaphigera* (50mg/kg,p.o.) + 40% ethanol
Group VI--- MeOH fraction of *Sterculia scaphigera* (25mg/kg,p.o.) + 40% ethanol
Group VII---MeOH fraction of *Sterculia scaphigera* (50mg/kg,p.o.) + 40% ethanol

**Histopathology of stomach sections in pyloric-ligation induced ulcer in rats**
Histopathological examinations when compared to the histoarchitecture of the stomach of Group I (normal) animals, stomach of Group II rats (exposed to pyloric-ligation) revealed extensive damage of mucosa, characterized by loss of gland architecture with erosion of the epithelial layer and infiltration by inflammatory cells. In Group IV,V,VI&VII rats (exposed to MESS & MFSS and pyloric-ligation), only minimal disruption of mucosa was observed which is similar to that of Group III rats (exposed to ranitidine and pyloric-ligation). (Fig.1)

**Histopathology of stomach sections in ethanol induced ulcer in rats**
Histopathological examinations when compared to the histoarchitecture of the stomach of Group I (normal) animals, stomach of Group II rats (exposed to ethanol) revealed extensive damage of mucosa, characterized by loss of mucus and chief cells. In Group IV,V,VI&VII rats (exposed to MESS & MFSS and ethanol), only minimal disruption of mucosa was observed which is similar to that of Group III rats (exposed to ranitidine and ethanol). (Fig.2)

**Table 1. Effect of *Sterculia scaphigera* methanolic extract & methanolic fraction on pyloric-ligation induced ulcers in rats**

<table>
<thead>
<tr>
<th>Treatment Groups (Dose in mg/kg,i.p.)</th>
<th>Total gastric content (ml)</th>
<th>pH</th>
<th>ml of NaOH reqd.</th>
<th>Total acidity [mEq/L]</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control [pyloric-ligation]</td>
<td>4.38±0.224</td>
<td>3.143±0.32</td>
<td>5.929±0.322</td>
<td>59.29±3.22</td>
<td>4.714±0.285</td>
</tr>
<tr>
<td>RTD (10)</td>
<td>3.5±0.178 *</td>
<td>4.8±0.184*</td>
<td>3.943±0.17*</td>
<td>39.43±1.68*</td>
<td>3.143±0.39*</td>
</tr>
<tr>
<td>RTD (10) p.o.</td>
<td>3.61±0.128*</td>
<td>4.64±0.179*</td>
<td>3.786±0.09*</td>
<td>37.86±0.88*</td>
<td>2.643±0.24*</td>
</tr>
<tr>
<td>MESS (50)</td>
<td>3.74±0.141*</td>
<td>5.14±0.142*</td>
<td>3.72±0.108*</td>
<td>37.29±1.08*</td>
<td>2.714±0.46*</td>
</tr>
<tr>
<td>MESS (50) p.o.</td>
<td>2.72±0.153*</td>
<td>5.0±0.154*</td>
<td>4.34±0.13*</td>
<td>43.43±1.25*</td>
<td>2.214±0.36*</td>
</tr>
<tr>
<td>MFSS (25) p.o.</td>
<td>3.32±0.130*</td>
<td>4.31±0.045*</td>
<td>4.314±0.05*</td>
<td>43.14±0.45*</td>
<td>2.857±0.18*</td>
</tr>
<tr>
<td>MFSS (50) p.o.</td>
<td>3.04±0.149*</td>
<td>3.97±0.035*</td>
<td>3.97±0.035*</td>
<td>39.7±0.35*</td>
<td>2.214±0.36*</td>
</tr>
<tr>
<td>F, 6, 28 =</td>
<td>12.38</td>
<td>14.71</td>
<td>25.10</td>
<td>25.10</td>
<td>7.43</td>
</tr>
<tr>
<td>P =</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values represent the mean of 5 rats ± SEM

* Statistically significant compared with control group (p <0.05)

RTD --- Ranitidine
MESS --- Methanolic Extract of *Sterculia scaphigera*
MFSS --- Methanolic Fraction of methanolic extract of *Sterculia scaphigera*
Table 2: Effect of methanolic extract & methanolic fraction of *Sterculia scaphigera* on ethanol induced ulcers in rats

<table>
<thead>
<tr>
<th>Treatment Groups (Dose in mg/kg.p.o.)</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.6±0.114</td>
</tr>
<tr>
<td>II</td>
<td>2.5 ± 0.316*</td>
</tr>
<tr>
<td>III</td>
<td>0.8 ± 0.122 #</td>
</tr>
<tr>
<td>IV</td>
<td>1.0 ± 0.158#</td>
</tr>
<tr>
<td>V</td>
<td>0.7 ± 0.122#</td>
</tr>
<tr>
<td>VI</td>
<td>1.2 ± 0.122#</td>
</tr>
<tr>
<td>VII</td>
<td>1.0 ± 0.223#</td>
</tr>
</tbody>
</table>

| F (6, 28) = | 11.89 |
| P =         | <0.05 |

Values represent the mean of 5 rats ± SEM

* Statistically significant compared with vehicle control group (p <0.05)
# Statistically significant compared with ethanol group (p <0.05)

Group II should be compared with Group I.
Groups III, IV, V, VI and VII should be compared with Group II.

Fig.1  Results of Histology : (Ulcer induced by pyloric-ligation)
Fig.1 Histopathological examination of Stomach sections in normal and different treatment groups along with pyloric-ligation.

Fig. A-- The vehicle treated rats exhibited stomach section showing normal gastric mucosa.

Fig B&C--Stomach sections of the rats with pyloric-ligation showed loss of gland architecture with erosion of the epithelial layer and infiltration by inflammatory cell.
Fig.D&E--The rats treated with Ranitidine (10mg/kg, i.p. & p.o. resp.) along with pyloric-ligation showed almost normal architecture of gastric mucosa.

Fig.F&G– The rats treated with methanolic extract of *Sterculia scaphigera* (50mg/kg, i.p. & p.o. resp.) along with pyloric-ligation showed almost normal architecture of gastric mucosa.

Fig.H&I– The rats treated with methanolic fraction of *Sterculia scaphigera* (25&50mg/kg, p.o. resp.) along with pyloric-ligation showed almost normal architecture of gastric mucosa.

**Fig.2 Results of Histology**: (Ulcer induced by administration of ethanol)

[Images of histological sections are shown, labeled as Fig. A to Fig. F.]
Fig. 2. Histopathological examination of Stomach sections in normal and different treatment groups along with administration of ethanol

Fig. A -- The vehicle treated rats exhibited stomach section showing normal gastric mucosa.

Fig. B & C -- Stomach sections of the ulcer induced rats with ethanol showed loss of mucus and chief cells.

Fig. D -- Stomach sections of rats treated with Ranitidine along with ethanol showed almost normal cellular architecture of gastric mucosa.

Fig. E & F -- Stomach sections of the rats treated with methanolic extract of *Sterculia scaphigera* (50 mg/kg, i.p. & orally, resp.) along with ethanol showed almost normal cellular architecture of gastric mucosa.

Fig. G & H -- Stomach sections of the rat treated with methanolic fraction of *Sterculia scaphigera* (25 & 50 mg/kg, orally resp.) along with ethanol showed almost normal cellular architecture of gastric mucosa.

**DISCUSSION AND CONCLUSION**

The results obtained in the present study show that the methanolic extract of *Sterculia scaphigera* and its methanolic fraction possess antioxidants & antiulcer activity. It has been demonstrated that many drugs and formulations having potent antioxidant action are effective in healing experimentally induced gastric ulcers\[^{17,18,19}\] (Dhuley, 1999; George et al., 1999; Goel and Sairam, 2002). Many plants exhibit efficient antioxidant properties owing to their phenolic constituents. Most of tannins and flavonoids are phenolic compounds and are responsible for antioxidant properties of many plants\[^{20}\] (Larson, 1988). The DPPH assay is based on the ability of 1,1-diphenyl-2 picryl-hydrazyl a stable free radical, to decolorize in the presence of antioxidants. The DPPH contains an odd electron which is responsible for absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron
donated by an antioxidant compound, it is decolorized which can be quantitatively measured from the change in absorbance. That is why with the increasing concentration of extract % scavenging activity also increases.

Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors\(^{21}\) (Hoogerwerf and Pasricha, 2006). Consequently, reduction of gastric acid production as well as re-inforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result, more and more drugs, both herbal and synthetic are coming up offering newer and better options for treatment of peptic ulcer. The type of drugs varies from being proton-pump inhibitor to H2 antagonist or a cytoprotective agent. At the same time, each of these drugs confers simpler to several side effects like arrhythmias, impotence, gynaecomastia, enterochromaffin-like cell (ECL), hyperplasia and haemopoeitic changes\(^{22}\)(Akthar et al., 1992).

The present study showed that the methanolic extract of *Sterculia scaphigera* and its methanolic fraction possess gastroprotective activity as evidenced by its significant inhibition in the formation of ulcers induced by physical (pyloric-ligation ) and chemical factors (ethanol). Pylorus ligation-induced ulcers are due to autodigestion of the gastric mucosa and break down of the gastric mucosal barrier\(^{23}\) (Sairam et al., 2002).

The incidence of ethanol-induced ulcers developing predominantly in the glandular part of stomach was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products\(^{24}\)(Oates and Hakkinen,1988); and reactive oxygen species\(^{25}\)(Mizui et al., 1987) resulting in the damage of rat gastric mucosa\(^{26}\) (Peskar et al., 1986).

Ethanol-induced depletion of gastric wall mucus has been prevented by *Sterculia scaphigera*.

In rat, ulcerprotective activity of *Sterculia scaphigera* is quite similar to Ranitidine [H2-antagonist], a reference ulcerprotective agent. Both of them improved the parameters of pyloric-ligation and ethanol induced ulcer including total acidity, volume of acid secretion, pH of gastric content and ulcer index.

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The antiulcer activity of various polysaccharides in experimental ulcers has also been reported\cite{27,28} (Sun et al., 1992; Matsumoto et al., 2002).

*Sterculia scaphigera* significantly ($P < 0.01$) reduced the ulcer index and afforded significant protection against ethanol-induced ulcer. The antioxidant properties of *Sterculia scaphigera* may have scavenged the free radicals produced by the metabolism of ethanol and thereby heal the ulcers.

Ethanol-induced gastric lesion formation may be due to stress in gastric blood flow that contributes to the development of the hemorrhage and necrotic aspects of tissue injury\cite{29} (Guth et al., 1984). This chemical agent also increases Na+ and K+ flux into the lumen and increases pepsin secretion along with histamine release.

Ethanol induces oxidative stress in rat gastrointestinal mucosa, increasing lipid peroxidation and DNA fragmentation and leading to gastric lesions. It also depresses tissue levels of DNA, RNA and proteins, altering blood flow, producing necrosis and hemorrhage- like gastric tissue\cite{30} (Kwiecien et al., 2002).

The antiulcerogenic potential of *Sterculia scaphigera* was further evidenced by the histopathological studies (Fig.1 and Fig.2) The Qualitative phytochemical investigations of Methanolic extract & Methanolic fraction of *Sterculia scaphigera* have shown the presence of alkaloids and flavonoids. Methanolic extract & fraction of *Sterculia scaphigera* offers protective effect against pyloric ligation & ethanol induced ulcer in experimental rats. The mechanism of action is yet to be investigated but may be due to the antioxidant effects found to be present in the extract.

Hence, it can be suggested that the antiulcer activity of the extract may be attributed to its antisecretory and antioxidant activities.
REFERENCES

