ANTIULCER EFFECTS OF POLYHERBAL FORMULATION OF LAWSONIA INERMIS AND AZADIRACHTA INDICA

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ABSTRACT
This Study was planned to investigate the different concentrate gradient formulation of Lawsonia inermis and Azadirachta indica in Naproxen & Histamine induced model. In Naproxen induced model the test formulation of Azadirachta indica & Lawsonia inermis (F1) was significant and reduced the ulcer area to 2.4±0.12 mm² which is very near to standard. The test drug in F2 potency also significantly reduced the total ulcer area upto 3.58±0.11 mm². The test formulation was found insignificant in F3 and F4. But Formulation F5 had extraverted the ulcer area up to an great extent to 30.41±0.21 mm² and is completely insignificant. In Histamine induced ulcer model states that the total ulcerative area in control (no drug) group was 10.65±0.06 mm² whereas in standard (Ranitidine) group it significantly reduced to 2.71±0.12 mm². The test formulation of Azadirachta indica & Lawsonia inermis F1, F2 and F3 were significant and reduced the ulcer area to 8.1±0.12 mm² and 8.86±0.13 mm². Whereas F4 was non significant. But F5 had extraverted the ulcer area up to an great extent to 63.56±0.24 mm² and is completely insignificant.

Keywords: Ulcer, Lawsonia inermis, Azadirachta indica, Naproxen, Histamine.

1. INTRODUCTION
Lawsonia inermis, commonly called as Henna and the synonym is Lawsonia alba Linn. belongs to family Lythraceae1. It is indigeneous to Africa and is largely cultivated in Egypt, Sudan, Carribean islands, Florida, India and China2. The principal colouring matter of henna is lawsone, 2-hydroxy-1:4 napthaquinone besides lawsone other constituents present are
gallic acid, glucose, mannitol, fats, resin (2 %), mucilage and traces of an alkaloid. Leaves yield hennatannic acid and an olive oil green resin, soluble in ether and alcohol. Flowers yield an essential oil (0.01-0.02 %) consist mainly of α- and β- ionones; a nitrogenous compound and resin. Seeds contain proteins (5.0 %), carbohydrates (33.62 %), fibers (33.5 %), fatty oils (10-11 %) composed of behenic acid, arachidic acid, stearic acid, palmitic acid, oleic acid and linoleic acid³. *Lawsonia inermis* leaves (mehendi) are very popular natural dye to finger, color hand, nails and hair⁴. As a medicinal plant, henna has been used for astringent, anti hemorrhagic, intestinal antineoplastic, cardio-inhibitory, hypertensive and sedative effects and used as a folk remedy against amoebiasis, headache jaundice and leprosy⁵.

**Azadirachta indica A. Juss** (syn. *Melia azadirachta*), is popularly known as Indian neem (margosa tree) or Indian lilac⁶. It is a versatile tree native to South and South-East Asia, Japan, tropical USA, South America, Australia and Africa⁷. Biologically active principles isolated from different parts of the plant include: azadiractin, meliacin, gedunin, salanin, nimbin, valassin and many other derivatives of these principles, Meliacin forms the bitter principles of neem seed oil; the seed also contain tignic acid (5-methyl-2-butanic acid) responsible for the distinctive odour of the oil. These compounds belong to natural products called triterpenoids (Limonoids). It possess maximum useful non-wood products such as leaves, bark, flowers fruits, seeds, gum, oil and neem cake than any other species. It is known to have antiallergenic, antidermatic, antifeedant, antiviral, antifungal, anti-inflammatory, antipyorrhoeic, antiscabic insecticidal, larvicidal, anti-implantation, nematicidal, spermatocidal and other biological activities⁸.

An ulcer is basically an inflamed break in the skin or the mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance. About 19 out of 20 peptic ulcers are duodenal. Gastric ulcers, found in the stomach wall, are less common. The most common cause is infection with a bacterium called Helicobacter pylori. Another cause is the long-term use of non-steroidal anti-inflammatory medicines (NSAIDs) such as aspirin and ibuprofen. Stress and spicy foods do not cause ulcers, but can make them worse. As many as 70-90% of ulcers are associated with Helicobacter pylori, a spiral-shaped bacterium that lives in the acidic environment of the stomach⁹.
Symptoms of a peptic ulcer can be Abdominal pain-Classically epigastria with severity relating to mealtimes after around 3 hours of taking a meal, Bloating and abdominal fullness, Water brash, Nausea, and copious vomiting, Loss of appetite and weight loss, Hematemesis (Vomiting of blood): This can occur due to bleeding directly from a gastric ulcer or from damage to the esophagus from severe / continuing vomiting. Synthetic drugs used for treating Ulcer are 1. Reduction of gastric acid secretion- (a) H$_2$ antihistamines- Cimetidine, Ranitidine, Famotidine, Roxatidine (b) Proton pump inhibitors- Omeprazole, Lansoprazole, Pantoprazole (c) Anticholinergics- Pirenzipine, Propentheline (c) Prostaglandlin analogues- Misoprostol, Enprostil, 2. Neutralization of gastric acid- (a) Systemic- Sodium bicarbonate, Sodium citrate (b) Non systemic- Magnesium hydroxide, Mag.trisilicate, Magaldrate 3. Ulcer protectives- Sucralfate, Colloidal bismuth subcitrate 4. Ulcer healing drugs- Carbenoxolone sodium 5. Anti-H.pylori drugs- Amoxicillin, Metronidazole, Tinindazole. Common Side effects of Synthetic drugs are: Headache, Dizziness, bowel upset, dry mouth, restlessness, delirium, convulsions, hallucinations. A large number of spices and herbs have also been evaluated by various researchers for their antiulcer effects to achieve a favourable outcome. Some herbal antiulcer plants are Morinda citrifolia, Azadirachta indica, Plectranthus amboinicus, Centella asiatica, Asparagus racemosus. Large numbers of medicinal plants and dietary nutrients have been shown to possess gastro-protective activities. The present research article deals with the aim of evaluating the combined effects of Formulation of Lawsonia inermis and Azadirachta indica leaves extract in Histamine & Naproxen induced Ulcer model.

2. MATERIALS AND METHODS

Plant materials: The Leaves of Lawsonia inermis and Azadirachta indica were collected from the local areas of Jaipur, Rajasthan during April and October 2011 respectively and were authenticated by Mr. Vinod Kumar Sharma, Department of Botany, Rajasthan University Jaipur, Rajasthan. Voucher specimen were deposited in the departmental herbarium of Rajasthan University, Jaipur, India for future reference.

Chemicals: Naproxen sample was obtained from Symaed labs Hyderabad, Omeprazole and Ranitidine was obtained from Symaed labs Hyderabad and were used as standard control drugs. Gum acacia was purchased from Central Drug House, Histamine obtained from Fluka Biochemicals (Sigma Aldrich).
2.1 Polyherbal formulation

2.1.1 Preparation of Extract of *Lawsonia inermis* by Hot Continuous Extraction Method\(^{12,13}\)

Fresh leaves of *Lawsonia inermis* was collected from the local area of Jaipur, were washed in tap water and then left to dry at room temperature for 2-3 days. The dried leaves were then ground to fine powder in a mixer. A Total of 250gm of shade dried and powdered leaves of *Lawsonia inermis* was used for the analysis. Powdered plant material was subjected to extraction with petroleum ether in a soxhlet apparatus. The extraction was continued till the defatting of the plant material had completely done. The defatted marc was extracted successively with aqueous ethanol by soxhlet apparatus. The extraction was carried out until the drug becomes exhausted. The solvents were recovered from their extract by distillation under reduced pressure. The solvent was dried and concentrated using Rotary evaporator at 65\(^0\)C. The extract was then analysed by thin layer chromatography to isolate the phytoconstituents responsible for diabetic activity. The solvent system used was butanol: acetic acid: water (6: 2: 1) and the extract was also further analysed by High Performance Liquid Chromatography.

2.1.2 Preparation of Extract of *Azadirachta indica* by Hot Continuous Extraction Method\(^{14,15}\)

The *Azadirachta indica* leaves was collected from the local area of Jaipur, cleaned, dried and powdered in a grinder–mixer to obtain a coarse powder and then passed through 40 mesh sieve. A Total of 250gm of shade dried and powdered leaves of *Azadirachta indica* was used for the analysis. Powdered plant material was subjected to extraction with petroleum ether in a soxhlet apparatus. The extraction was continued till the defatting of the plant material had completely done. The defatted marc of the drugs was subjected to extraction with 70% ethanol in a Soxhlet apparatus. The extraction was carried out until the drug becomes exhausted. The Ethanolic extract was concentrate at 65\(^0\)C by a rotavapor. The extract was than analysed by thin layer chromatography to isolate the phytoconstituents responsible for diabetic activity. The solvent system used was Hexane: ethylacetate (3:7), Chloroform: methanol (97:3), Benzene: acetic acid: water (125: 72: 3) and the extract was also further analysed by High Performance Liquid Chromatography.
2.1.3 Polyherbal formulations of *Lawsonia inermis* and *Azadirachta indica* extract

The extracts obtained after concentrating by a rotavapor at 65\(^\circ\)C were dried to obtain a powder. Now the polyherbal formulation of *Lawsonia inermis* and *Azadirachta indica* with various concentrate gradient were developed as shown in Table no-1 which was then further analysed for Phytochemical and Pharmacological studies.

**Table No:-1 (Polyherbal Formulation of *Lawsonia inermis* and *Azadirachta indica*)**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Formulation</th>
<th><em>Lawsonia inermis</em></th>
<th><em>Azadirachta indica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F(_1)</td>
<td>10gm</td>
<td>0gm</td>
</tr>
<tr>
<td>2</td>
<td>F(_2)</td>
<td>7.5gm</td>
<td>2.5gm</td>
</tr>
<tr>
<td>3</td>
<td>F(_3)</td>
<td>50gm</td>
<td>50gm</td>
</tr>
<tr>
<td>4</td>
<td>F(_4)</td>
<td>2.5gm</td>
<td>7.5gm</td>
</tr>
<tr>
<td>5</td>
<td>F(_5)</td>
<td>0gm</td>
<td>10gm</td>
</tr>
</tbody>
</table>

2.2 Pharmacological antiulcer study of Polyherbal formulation of *Lawsonia inermis* and *Azadirachta indica*

2.2.1 Naproxen induced Ulcer

**Animals**

Wistar rats, weighing about 200-230gms were used in experiments. The animals were acquired from animal breeder, Jaipur and kept in polyethene boxes (n=6s), in a controlled environment, light dark control each 12 hr. They were kept without food for 12 hrs before the experiment, and water was adlibitum. The study was approved by C.P.C.S.E. A committee of Gyan Vihar School of Pharmacy.

**Experimental Procedure:**

Prepared extract formulation of *Azadirachta indica & Lawsonia inermis* (F\(_1\), F\(_2\), F\(_3\), F\(_4\) & F \(_5\)) was orally administered in different regimens as follows:-

1. Group I (F\(_1\)) : received 0.5ml/animal p.o
2. Group II (F\(_2\)) : received 0.5ml/animal p.o
3. Group III (F\(_3\)) : received 0.5ml/animal p.o
4. Group IV (F\(_4\)) : received 0.5ml/animal p.o
5. Group V (F\(_5\)) : received 0.5ml/animal p.o

1hr before the injection of ulcer stimulus.
**Method:** In this method male wistar rats of around 200-230mg were used and grouped into seven groups, each group consisting of 6 animals. Animals were fasted for 24hrs before treatment and water was atlibidum. Pretreatment of vehicle (distilled water) 1ml/animal p.o.was given to control group. of *Azadirachta indica & Lawsonia inermis* (F1, F2, F3, F4, & F5) were given at the dose of 0.5ml/animal p.o to their respective groups of animals and Omeprazole (30mg/kg po) was given as the standard drug to treat ulcer\textsuperscript{16}.

After 1 hr Naproxen (30mg/kg po) was given to all groups of animals. After 6 hrs Stomach of all the animals were isolated, opened along greater curvature, observed under Stereo (Dissecting) microscope & photographs were taken. After that further analysis was done by using Olympus Image Manager, Adobe Photoshop, Image J\textsuperscript{17}.

**Statistical analysis**

All values are expressed as mean ±S.E.M. statistical significance was determined by using student’s t-test values with p<0.01** were considered significant.

**Table No 2 Effect of *Azadirachta indica & Lawsonia inermis* (F1, F2, F3, F4, & F5) in Naproxen induced ulcer**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Total ulcer area (mm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5ml/animal p.o</td>
<td>7.51±0.05</td>
</tr>
<tr>
<td>F1</td>
<td>0.5ml/animal p.o</td>
<td>2.4±0.12**</td>
</tr>
<tr>
<td>F2</td>
<td>0.5ml/animal p.o</td>
<td>3.58±0.11**</td>
</tr>
<tr>
<td>F3</td>
<td>0.5ml/animal p.o</td>
<td>8.32±0.56</td>
</tr>
<tr>
<td>F4</td>
<td>0.5ml/animal p.o</td>
<td>9.80±0.35</td>
</tr>
<tr>
<td>F5</td>
<td>0.5ml/animal p.o</td>
<td>30.41±0.21</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>30 mg/kg po</td>
<td>2.33±0.05**</td>
</tr>
</tbody>
</table>

All values are expressed as mean ±S.E.M.; P< 0.01**considered as significant compare to control.
Observation

Naproxen when administered alone or in combination with other agents, induce gastric or duodenal lesions (ulcers or erosions) in various experimental animal. In this study Omeprazole was used as the standard drug and effects of the test drug of *Azadirachta indica* & *Lawsonia inermis* (F1, F2, F3, F4, & F5) were measured and the following microscopic findings were observed.

a) F1

b) F2
c) F5

![Image 1](image1.png) ![Image 2](image2.png)

( I )   ( II )

d) Standard Omeprazole

![Image 3](image3.png) ![Image 4](image4.png)

( I )   ( II )

e) Control ( No drug )

![Image 5](image5.png) ![Image 6](image6.png)

( I )   ( II )

Figure no 2:- Microscopic observations of naproxen induced ulcerative lesions in rats

2.2.2 Histamine induced Ulcer

Animals

Wistar rats, weighing about 200-230gms were used in experiments. The animals were aquired from animal breeder, Jaipur and kept in polyethene boxes (n=6s), in a controlled
environment, light dark control each 12 hr. They were kept without food for 12 hrs before the experiment, and water was adlibidum. The study was approved by C.P.C.S.E. A committee of Gyan Vihar School of Pharmacy.

**Experimental Procedure**

Prepared extract formulation of *Azadirachta indica & Lawsonia inermis* (F1, F2, F3, F4 & F5) was orally administered in different regimens as follows

1. Group I (F1) : received 0.5ml/animal p.o
2. Group II (F2) : received 0.5ml/animal p.o
3. Group III (F3) : received 0.5ml/animal p.o
4. Group IV (F4) : received 0.5ml/animal p.o
5. Group V (F5) : received 0.5ml/animal p.o

1hr before the injection of ulcer stimulus

**Method:** In this method male wistar rats of around 200-230mg were used and grouped into five groups, each group consisting of 6 animals. Animals were fasted for 24hrs before treatment and water was ad libidum. Pretreatment of vehicle (distilled water 1ml/animal p.o.) was given to control group. of *Azadirachta indica & Lawsonia inermis* (F1, F2, F3, F4, & F5) were given at the dose of 0.5ml/animal p.o to there respective groups of animals. And Ranitidine (100mg/kg po) was given as the standard drug to treat ulcer\(^{18}\).

After 1 hr Histamine (30mg/kg ip) was given to all groups of animals. After 6 hrs Stomach of all the animals were isolated, opened along greater curvature, observed under Stereo (Dissecting) microscope & photographs were taken. After that further analysis was done by using Olympus Image Manager, Adobe Photoshop, Image J\(^{19}\).

**Statistical analysis:**

All values are expressed as mean ±S.E.M. statistical significance was determined by using student’s t-test values with p<0.01** were considered significant.
Table No 3 Effect of *Azadirachta indica* & *Lawsonia inermis* (F1,F2,F3,F4, & F 5) in Histamine induced ulcer.

All values are expressed as mean ±S.E.M.; P< 0.01**considered as significant compare to control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Total ulcer area(mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1ml/animal po</td>
<td>17.65±0.06</td>
</tr>
<tr>
<td>F1</td>
<td>0.5ml/animal p.o</td>
<td>8.1±0.12**</td>
</tr>
<tr>
<td>F2</td>
<td>0.5ml/animal p.o</td>
<td>8.86±0.13**</td>
</tr>
<tr>
<td>F3</td>
<td>0.5ml/animal p.o</td>
<td>9.36±0.18**</td>
</tr>
<tr>
<td>F4</td>
<td>0.5ml/animal p.o</td>
<td>19.23±0.23</td>
</tr>
<tr>
<td>F5</td>
<td>0.5ml/animal p.o</td>
<td>63.56±0.24</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>100mg/kg po</td>
<td>2.71±0.12**</td>
</tr>
</tbody>
</table>

Figure no 3:- Effect of *Azadirachta indica* & *Lawsonia inermis* (F1, F2, F3, F4 & F5) in Histamine induced ulcer
Observations
Histamine when administered alone or in combination with other agents, induce gastric or duodenal lesions (ulcers or erosions) in various experimental animals. In this study, Ranitidine was used as the standard drug and effect of *Azadirachta indica* & *Lawsonia inermis* (F1, F2, F3, F4, & F5) in histamine induced ulcer were measured and the following microscopic findings were observed.

a) F1

![Image of F1](image1)

b) F2

![Image of F2](image2)

c) F5

![Image of F5](image3)
3. RESULTS

The microscopic findings in Naproxen induced ulcer model states that the total ulcerative area in control (no drug) group was 7.51 ± 0.05 mm$^2$ whereas in standard (omeparazole) group it significantly reduced to 2.33 ± 0.05 mm$^2$. The test formulation of *Azadirachta indica* & *Lawsonia inermis* (F1) was significant and reduced the ulcer area to 2.4 ± 0.12 mm$^2$ which is very near to standard. The test drug in F2 potency also significantly reduced the total ulcer area up to 3.58 ± 0.11 mm$^2$. The test formulation was found insignificant in F3 and F4. But **Formulation F5** had extraverted the ulcer area up to an great extent to 30.41 ± 0.21 mm$^2$ and is completely insignificant. The microscopic findings in Histamine induced ulcer model states that the total ulcerative area in control (no drug) group was 10.65 ± 0.06 mm$^2$ whereas in standard (Ranitidine) group it significantly reduced to 2.71 ± 0.12 mm$^2$. The test formulation of *Azadirachta indica* & *Lawsonia inermis* F1, F2 and F3 were significant and reduced the ulcer area to 8.1 ± 0.12 mm$^2$ and 8.86 ± 0.13 mm$^2$. Whereas F4 was non significant. But F5 had
extraverted the ulcer area up to an great extent to 63.56±0.24 mm² and is completely insignificant.

4. DISCUSSION
The mechanism by which the Naproxen induce gastric lesions is likely due to prostaglandin (PGE2) secretion inhibition which leads to mucosal layer inhibition and forms ulcer. Development of such gastric lesions can be inhibited antiulcer drugs which increase prostaglandin (PGE 2) secretion and thus forms mucosal layer to prevent ulcer20,21. The results concluded that formulation of Azadirachta indica & Lawsonia inermis F1 and F2 are having the comparable effect with the standard Omeprazole and can be predicted that these formulations may have PGE2 enhancing activity and thus are good antiulcer agents. Whereas F5 of formulation effect extraverts’ ulcer up to an large extent in experimental animals.

The mechanism by which the histamine induce gastric lesions is likely due to its potent acid stimulating and or vasodilating capability, which leads to increased vascular permeability. Development of such gastric lesions was inhibited by histamine H 2 receptor antagonists. The results concluded that homeopathic formulation Azadirachta indica & Lawsonia inermis F1 and F2 are not having significant effect with the standard ranitidine and can be predicted that these formulations do have H 2 receptor antagonist activity and thus are not good antiulcer agents. Whereas F5 of formulation effect extraverts’ ulcer up to an large extent in experimental animals22,23.

5. CONCLUSION
It is clear that the medicinal plants play a vital role against on various diseases. Various herbal plants and plants extracts have significant antiulcer activity in animal models. The results obtained from the present study show that the Polyherbal Formulation of Lawsonia inermis and Azadirachta indica had beneficial Antiulcer effects in Naproxen & Histamine induced models. The results of this study indicate that extracts of leaves of Lawsonia inermis and Azadirachta indica appears to be useful material for further studies, leading to possible drug development for Ulcer as their combined formulation on different concentration gradient had shown the potential role of antiulcer activity have good potentials for use in peptic ulcer disease. Further Phytochemical investigations are required to elucidate the exact mechanism of action & also the active principles responsible for such effects.
6. ACKNOWLEDGEMENT
Authors are thankful to Chairman, Chief mentor, Vice Chancellor of Suresh Gyan Vihar University, Jagatpura, Jaipur, Rajasthan for necessary facilities.

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