Polyherbal mixture Protects against Ethanol-Induced Gastric Ulcers and liver necrosis in Rats.

Kulkarni A. S., 1* C. Vijaya Raghavan, 2 Patil D.M., 3
1RVS College of Pharmaceutical Sciences Sulur, Coimbatore, Tamilnadu, India
2PSG College of Pharmacy, Tamilnadu, India
3RVS College of Pharmaceutical Sciences Sulur, Coimbatore, Tamilnadu, India

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ABSTRACT

Background: Polyherbal mixture; containing a group of Flavonoids, tannins, vitamins, steroids etc. Objectives: The aim of this study was to examine the effects of administration of polyherbal mixture on ethanol-induced gastric ulcers and liver necrosis in conscious rats. Methods: Ethanol and Polyherbal mixture was administered orally for 21 days; mucosal lesions and liver histopathology were examined microscopically. Results: Polyherbal mixture 400mg/kg dose-dependently reduced, ethanol-induced gastric ulcers by 75% as well as liver cells were also regenerated. Conclusions: The gastric mucosal protection and liver cell regeneration against ethanol can be mediated through a number of mechanisms that include enhancement of the gastric mucosal defense through increase in mucus decrease in gastric acid secretion or simply neutralized gastric acidity and recover the hepatic damage cell.

Keywords: Polyherbal mixture, gastric ulcer, hepatotoxicity, regeneration

INTRODUCTION

There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Polyherbal Mixture contains five drugs like Trichosanthes dioica, Pistacia khinjuk, Cheilanthes albomarginata, Cheilanthes farinose and Clematis gouriana. All these drugs are have pharmacological properties like lipid lowering, in hyperglycemia, asthma, gastro-intestinal disorders1 skin diseases2 anti-inflammatory and anti-nociceptive3,4 anthelmintic5 antioxidant, antimicrobial6,7, anti-inflammatory, antipyretic, antibacterial, antiviral, in treatment diarrhoea and throat infection8,9,10. The plant has a root like stem which is a creeping rhizome and grows in a variety of situations particularly along water streams and near waterfalls. The powdered fronds of Cheilanthes farinosa, mixed with butter are used for the treatment of eczema, scabies and various other skin disorders which might result in lesions and wounds 6. The aerial parts from this genus are particularly used in Asia and Europe as a remedy to reduce pain and fever, as diuretic, in the treatment of rheumatic pain, eye infections, gonorrhoeal symptoms, bone illnesses, chronic skin disorders, gout and varicosis7,8. One of the species C. gouriana from the genus is found in India. It is not yet much evaluated for its phytochemical constitution and pharmacological activities. This species is found in the Western Ghats of the India. Clematis gouriana Roxb. is a climbing shrub, from the family Ranunculaceae which is commonly known as the “Indian traveller’s joy” 9. The fruits are tonic and stomachic. The roots of C. gouriana are the traditionally used in cardiovascular disease10. And the leaves of C. gouriana are recommended in folk medicine as the antipyretic with wound healing properties and in skin diseases. Trichosanthes dioica Roxb being very rich in protein and vitamin A, it has certain medicinal properties, and many reports are available regarding its role in lowering of blood sugar and serum triglycerides11. The fruits are easily digestible and diuretic in nature. They are also known to have antiulcer effects it grows as a vegetable all over India. The powdered fronds of Cheilanthes farinosa, mixed with butter are used for the treatment of eczema, scabies and various other skin disorders which might result in lesions and wounds. The juice of the leaf is applied to patches of alopecia areata 12,13. The root is used as a hydragogue cathartic, tonic and febrifuge.14

Methods

Preparation of polyherbal mixture

The leaves, fruits and stems of Trichosanthes dioica, Pistacia khinjuk, Cheilanthes albomarginata, Cheilanthes farinose and Clematis gouriana were shade dried at room temperature. The dried aerial parts were then crushed with anesthetic ether and blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary tube and collected in plain sterile centrifuge tubes and allowed to clot. Serum was separated by centrifugation at 10000 rpm for 15 min. at 5°C. The separated serum was used for estimation of AST, ALT, ALP, TG and TB.

Animal:

All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the polyherbal drug mixture of Control and Supervision on Experimentation on Animals (1012/c/06/ CPCSEA) and were approved by the Institutional Animal Ethics Committee. Wistar rats weighing between 180 to 200 gm of either sex were used. Animals had free access to standard pellet diet and water ad libitum.

Biochemical Estimations:

On the day 22 after overnight fasting of animals all the animals were anesthetized with anesthetic ether and blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary tube and collected in plain sterile centrifuge tubes and allowed to clot. Serum was separated by centrifugation at 10000 rpm for 15 min. at 5°C. The separated serum was used for estimation of AST, ALT, ALP, TG and TB.
Histopathological Investigations:
On the day 22nd the animals were sacrificed and abdomen was cut open, the liver was dissected out. The liver was processed for the histopathological investigations.

METHODS

Ethanol-Induced Gastric Ulcers and liver necrosis in Rats

Treatment schedule: 21
Group I: -The animals in this group received distilled water (1ml/100gm body weight, p.o.) as vehicle from day 1 to day 21. Group II: - The animals in this group received ethanol (3ml/100gm body weight, p.o.) from day 1 to day 21 in two divided doses and Silymarin (100 mg/kg, p.o.) from day 1 to day 21. Group IV: - The animals in this group received ethanol (3ml/100gm body weight, p.o.) from day 1 to day 21 in two divided doses and Polyherbal mixture (100mg/kg, p.o.) From day 1 to day 21. Group V: - The animals in this group received ethanol (3ml/100gm body weight, p.o.) from day 1 to day 21 in two divided doses and Polyherbal mixture (200mg/kg, p.o.) from day 1 to day 21. Group VI: - The animals in this group received ethanol (3ml/100gm body weight, p.o.) from day 1 to day 21 in two divided doses and Polyherbal mixture (400mg/kg, p.o.) from day 1 today 21.

Group I: Normal control group. Group II: Negative control / Ethanol treated group. Group III: Positive control / Silymarin. Group IV: Polyherbal mixture100 mg/kg, p.o. Group V: Polyherbal mixture200 mg/kg, p.o. Group VI: Polyherbal mixture 400 mg/kg, p.o. P.O.: per oral

Table. II : Effect of Polyherbal Mixture in Ethanol Induced Gastric Ulcer in Rats.

<table>
<thead>
<tr>
<th>Table II : Effect of Polyherbal Mixture in Ethanol Induced Gastric Ulcer in Rats.</th>
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</thead>
<tbody>
<tr>
<td><strong>Sr No</strong></td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>AST (U/ml)</td>
</tr>
<tr>
<td>ALT (U/ml)</td>
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<tr>
<td>ALP (U/ml)</td>
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<tr>
<td>TG (mg/dl)</td>
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<tr>
<td>¹B (mg/dl)</td>
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<tr>
<td>V Omeprazole</td>
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</tbody>
</table>

Table. II : Effect of Polyherbal Mixture in Ethanol Induced Gastric Ulcer in Rats.

<table>
<thead>
<tr>
<th><strong>Group</strong></th>
<th><strong>Treatment</strong></th>
<th><strong>Ulcer Index</strong></th>
<th><strong>Percentage Inhibition</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>25.76 ±3.12</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Polyherbal Mixture 100</td>
<td>14.26 ± 1.35*</td>
<td>44.64</td>
</tr>
<tr>
<td>III</td>
<td>Polyherbal Mixture 200</td>
<td>10.58 ± 0.60**</td>
<td>58.92</td>
</tr>
<tr>
<td>IV</td>
<td>Polyherbal Mixture 400</td>
<td>7.82 ± 1.43**</td>
<td>69.64</td>
</tr>
<tr>
<td>V</td>
<td>Omeprazole</td>
<td>4.30 ± 1.61**</td>
<td>83.3</td>
</tr>
</tbody>
</table>

n=6. Values are expressed as Mean ± S.E.M.
* = p < 0.05, ** = p < 0.01 when compared with Negative control,
### * = p < 0.01, when compared with normal control,

Statistically analyzed by One Way ANOVA followed by Dunnett test.

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Group I: treated with 4% v/v aqueous tween 80 (10 ml/kg p.o), Group II: treated with polyherbal mixture 100 mg/kg p.o) respectively for 14 days., Group III: treated with polyherbal mixture 200 mg/kg p.o) respectively for 14 days., Group IV: treated with polyherbal mixture 400 mg/kg p.o) respectively for 14 days., Group V: treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 14th day, Gastric ulcers were induced with ethanol at a dose of 5ml/kg administered to all groups by orally. 22 The animals were anaesthetized 6 hrs. with ether and stomachs were incised along the greater curvature and the ulcer index for each rat was taken as the mean ulcer score.

Measurement of Ulcer Index

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant, if any. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%I) was calculated as described by using the following formula.

\[
\%I = \frac{(USc - USt) \times 100}{USc}
\]

Where USc = ulcer surface area in control

USt = ulcer surface area in treated animals

CONCLUSION

Ethanol is primarily metabolized by alcohol dehydrogenase with the formation of acetaldehyde. Several other pathways are cytochrome p-450 dependent microsomal ethanol-oxidizing system, catalase and non-enzymatic ethanol oxidation and the involvement of free radical species. Ethanol induced hepatic hypoxia is also one of the main causes of hepatotoxicity. 22 Ethanol increases triglycerides and cholesterol levels thus inducing imbalance in lipid metabolism in liver, heart and kidney. 24

The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membranes. In the present study, the transaminase level increased by chronic ethanol treatment was reduced by polyherbal mixture 400mg/kg.

ALP activity was related to functioning of the hepatocyte cells. This ALP level was reduced by significantly due to polyherbal mixture 400mg/kg. It also decreased serum triglycerides and cholesterol levels indicating its hepatoprotective activity. The total bilirubin level was also restored to the normal level polyherbal mixture 400mg/kg treatment. The result indicates that treatment with polyherbal mixture 400mg/kg protect liver against ethanol induced hepatotoxicity.

The antiulcer effect of polyherbal mixture was tested against gastric lesions with production of reactive oxygen species. Reactive oxygen species are involved in the pathogenesis of ethanol induced gastric mucosal injury in vivo. 22 The gastric mucosal protection against ethanol can be mediated through a number of mechanisms that include enhancement of the gastric barrier, restoration of mucus production, and inhibition of neutrophil infiltration. 22

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