Immunomodulatory and Wound Healing Activity of Aqueous Extract of Terminalia Tomentosa Barks

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Abstract—The aim of this study was to evaluate the bilirubin lowering and wound healing property of aqueous extract of Terminalia tomentosa (AECP) bark in Wistar rats. Albino Wistar rats of either sex were used for the study. Bilirubin lowering property of Terminalia tomentosa barks was evaluated using phenyl hydrazine and paracetamol as inducing agents followed by measuring the concentration of serum total bilirubin in hyperbilirubinemia rats. Wound healing property was evaluated using incision and excision models by measuring tensile breaking strength, percentage wound contractions, and epithelization days, respectively. Statistical Analysis: Statistical comparison between groups in each experiment was done with one-way analysis of variance followed by Dunnett’s test.

Index Terms—Terminalia tomentosa, excision, hyperbilirubinemia, incision, paracetamol, phenylhydrazine, wound healing.

I. INTRODUCTION

The human race started using plants or plant products successfully as a mean of treatment of disease and injuries as effective therapeutic tools from early days of civilization to the modern age. Terminalia tomentosa is an ayurvedic plant with important medicinal properties. Botanical Name: Terminalia tomentosa Common Name: Asan, Indian Laurel, Silver grey woodIt occurs frequently in Indonesia, Malaysia, China, India as wasteland weed and also found in most parts of the world with a warm climate in dry, sandy, and alkaline soils. Terminalia tomentosa is an erect, tall, highly branched, and perennial shrub or small tree that grows to a height of 5.4 m with milky latex throughout the plant. The photochemistry of plant reveals presence of tri terpenoids, flavonoids, cardiac glycosides, cardenolides, anthocyanins, α-amyrin, β-amyrin, lupeol, β-sitosterol, flavones, madarine, resins, a powerful bacteriolytic enzyme calactin, a nontoxic proteolytic enzyme calotropin, and a wax. The parts of plants used in Ayurveda medicine are leaves, fresh or dried, the roots, root bark, and flowers. The powdered leaves are useful for fast healing of wounds, as purgative, to treat liver problems, to promote sexual health, to relieve stomach ache, headache, also applied in sprain to ease swelling and pain. Traditionally, the plant has been used as anti-fungal, antipyretic, analgesic. The dried leaves are used as an expectorant, anti-inflammatory, for the treatment of paralysis and rheumatic pain. The dried latex and roots are used as an antidote for snake poisoning. It is also used as an abortifacient for the treatment of piles and intestinal worms. The tender leaves are also used to treat migraine. Therefore, by taking into limelight the traditional uses of Calotropis procera, the present study was performed to provide a pharmacological base for use of plant in treatment of hyperbilirubinemia induced by phenylhydrazine (PHZ) and paracetamol and wound healing.

II. MATERIALS AND METHODS

Animals: Adult Wistar rats of either sex weighing about 180–250 g were purchased from Bharat serum and vaccines, Thane. The animals were housed in standard laboratory conditions in groups of three at 25°C ± 2°C, humidity 60% ± 2%, and 12 h light: Dark cycle. Animals had a free access to standard laboratory food purchased from Amrut rat and mice feed, Nashik, India and water. The animals were acclimatized to the laboratory conditions 1 week prior to the experimentation. All experiments were performed during a light portion of 12–12 h. Drugs and Chemicals Phenylhydrazine (Sigma-Aldrich, USA), paracetamol (Merck), Silymarin (Silybon, Micro labs), Dexamethasone (DXM) injection (Life care pharmaceuticals), ketamine HCl (Aneket, Neon labs), Total Bilirubin kit, alanine transaminase (ALT), and aspartate transaminase (AST) (Coral Clinical Systems, Mumbai) were used in present study. Drug solutions were prepared fresh in distilled water and stored in a refrigerator at 4°C.

Preparation of Plant Extract: Barks of Terminalia tomentosa were collected from Tapovan garden, Panchvati, Nashik. The bark were authenticated by Dr. (Mrs.) A. G. Bhaskarwar, Ayurved Seva Sangh, Panchvati, Nashik. The collected barks were shade dried and powdered using a grinder. The aqueous extract of Terminalia tomentosa (AECP) was prepared by boiling the powdered bark matter with 16 times of its weight in distilled water and reducing its volume up to 1/32 times.
extract obtained was stored in refrigerator till used.

Phenylhydrazine Induced Hyperbilirubinemia: The animals were treated with PHZ (5 mg/kg i.p.) for 5 days to develop jaundice following standard procedure with slight modification. LD50 (993 mg/kg) was found to be reported in rats. Hence, the doses selected for study were 25 mg/kg and 50 mg/kg. The animals were randomly distributed into five groups (n = 5). Group I received vehicle (distilled water, 5 ml/kg, p.o.), Group II received PHZ (5 mg/kg, i.p.), Group III received PHZ (5 mg/kg, i.p.) and Silymarin suspension (100 mg/kg, p.o.), Group IV received PHZ (5 mg/kg, i.p.) and AECP (25 mg/kg, p.o.), and Group V received PHZ (5 mg/kg, i.p.) and AECP (50 mg/kg, p.o.). The concentration of serum total bilirubin was determined by Mod. Jendrassik and Grof’s method and hemoglobin (Hb) by Sahli–Hellige method on day 1 and day 5 after 6 h of administration of PHZ to confirm jaundiced condition of animals. The treatment of jaundiced groups with Silymarin (100 mg/kg, p.o.) and AECP (25 and 50 mg/kg, p.o.) was started on day 6 and continued up to day 10. Blood was collected from tail vein of rats on day 1 (normal), day 5 (after 6h of PHZ administration), and on day 10 to determine serum concentration of total bilirubin and Hb bark.

Paracetamol Induced Hyperbilirubinemia: The animals were treated with paracetamol (2 g/kg p.o.) for 5 days to develop jaundice following standard procedure with slight modification. The animals were randomly distributed into five groups (n = 5). Group I received vehicle (distilled water, 5 ml/kg, p.o.), Group II received paracetamol (2 g/kg p.o.), Group III received paracetamol (2 g/kg p.o) and Silymarin suspension (100 mg/kg p.o.), Group IV received paracetamol (2 g/kg p.o) and AECP (25 mg/kg, p.o.), Group V received paracetamol (2 g/kg p.o) and AECP (50 mg/kg, p.o.). The concentration of serum total bilirubin was determined by Mod. Jendrassik and Grof’s method, ALT and AST by Reitman and Frankel’s method[16] on day 1 and day 5 after 6 h of administration of paracetamol to confirm jaundice in animals. The treatment for jaundice with Silymarin (100 mg/kg, p.o.) and AECP (25 and 50 mg/kg, p.o.) was started on day 6 and continued up to day 10. Blood was collected from tail vein of rats on day 1 (normal), day 5 (after 6 h of paracetamol administration), and on day 10 to determine serum concentration of total bilirubin, ALT and AST.

Incision Wound Model: Animals were distributed into four groups (n = 5) as follows: Group I received vehicle (distilled water, 5 ml/kg, p.o.), Group II received DXM (0.34 mg/kg i.m. on 1st day and 0.17 mg/kg i.m. on alternative days for 7 days), Group III received AECP (25 mg/kg, p.o.), Group IV received AECP (50 mg/kg, p.o.). All procedures were carried out under ketamine anesthesia (60 mg/kg i.p.). On the depilated backs of Wistar rats, two paravertebral incisions of 2.5 cm were made cutting through the full thickness of the skin. Interrupted sutures, 1 cm apart were placed to approximate the cut edges of skin by ethilon 4-0. The sutures were removed after 7 days and skin breaking tensile strength was measured on day 10 by continuous water flow technique of Lee.

Excision Wound Model: Animals were randomly distributed into four groups (n = 5). Group I received vehicle (distilled water, 5 ml/kg, p.o.), Group II received DXM (0.34 mg/kg i.m. on 1st day and 0.17 mg/kg i.m. on alternative days till epithelization), Group III received AECP (25 mg/kg, p.o.), Group IV received AECP (50 mg/kg, p.o.), and received their respective treatment. An excision wound was inflicted by cutting away a circular piece of 0.5 mm to the full thickness of skin on a predetermined area on depilated back. Epithelization period was noted as the number of days required for Eschar to fall off leaving no raw wound behind. Wound contraction rate was monitored by measuring wound area on alternate days. Reduction in wound area expressed as a percentage of original wound size.

Statistical Analysis: All data expressed as mean ± standard error of mean (SEM) of value for corresponding parameters. Statistical comparison between groups in each experiment was performed with one-way analysis of variance followed by Dunnett’s test. Statistical analysis was performed using Primer software.

Results: Phenyl hydrazine Induced Hyperbilirubinemia Serum total Terminalia tomentosa barks Animals treated with PHZ (5 mg/kg i.p.) showed significant (P < 0.05) increase in serum bilirubin level on day 5 compared to day 1 and significant (P < 0.05) decrease in serum bilirubin on day 10 compared to day 5. Animals showed significant (P < 0.05) increase in the level of serum total bilirubin compared to vehicle treated group. Hyperbilirubinemia rats treated with Silymarin (100 mg/kg p.o.) and AECP (25 and 50 mg/kg p.o.) showed significant (P < 0.05) decrease in levels of serum total bilirubin as compared to PHZ treated group on day 10 of study [Table 1].Hemoglobin Animals treated with PHZ (5 mg/kg i.p.) showed significant (P < 0.05) decrease in Hb level on day 5 compared to day 1 and significant (P < 0.05) increase in Hb level on day 10 compared to day 5. Animals showed significant (P < 0.05) decrease in levels of Hb compared to vehicle treated group. Hyperbilirubinemia rats treated with Silymarin (100 mg/kg p.o) and AECP (25 and 50 mg/kg, p.o.) showed significant (P < 0.05) increase in levels of Hb as compared to the PHZ treated group on day 10 of study [Table 1].

Hemoglobin: Animals treated with PHZ (5 mg/kg i.p.) showed significant (P < 0.05) decrease in Hb level on day 5 compared to day 1 and significant (P < 0.05) increase in Hb level on day 10 compared to day 5. Animals showed significant (P < 0.05) decrease in levels of Hb compared to vehicle treated group. Hyperbilirubinemia rats treated with Silymarin (100 mg/kg p.o) and AECP (25 and 50 mg/kg, p.o.) showed significant (P <
0.05) increase in levels of Hb as compared to the PHZ treated group on day 10 of study [Table 1].

**Paracetamol Induced Hyperbilirubinemia Serum total Terminalia tomentosa:** Animals treated with paracetamol (2 g/kg p.o.) showed significant (P < 0.05) increase in serum bilirubin level on day 5 compared to day 1 and significant (P < 0.05) decrease in serum bilirubin on day 10 compared to day 5. Animals showed significant (P < 0.05) increase in the level of total bilirubin compared to vehicle treated group. Hyperbilirubinemic rats treated with Silymarin (100 mg/kg p.o.) and AECP (25 and 50 mg/kg p.o.) showed significant (P < 0.05) decrease in levels of serum total bilirubin as compared to paracetamol treated group on day 10 of study [Figure 1]. Animals treated with paracetamol (2 g/kg p.o.) showed significant (P < 0.05) increase in serum bilirubin level on day 5 compared to day 1 and significant (P < 0.05) decrease in serum bilirubin on day 10 compared to day 5. Animals showed significant (P < 0.05) increase in the level of total bilirubin compared to vehicle treated group. Hyperbilirubinemic rats treated with Silymarin (100 mg/kg p.o.) and AECP (25 and 50 mg/kg p.o.) showed significant (P < 0.05) decrease in levels of serum total bilirubin as compared to paracetamol treated group on day 10 of study [Figure 1].

**Serum alanine transaminase and aspartate transaminase bark:** Animals treated with paracetamol (2 g/kg p.o.) showed significant (P < 0.05) increase in serum level of ALT and AST on day 5 compared to day 1 and significant (P < 0.05) decrease in serum level of ALT and AST on day 10 compared to day 5. Animals showed significant (P < 0.05) increase in serum levels of ALT and AST compared to vehicle treated group. Hyperbilirubinemia rats treated with Silymarin (100 mg/kg p.o.) and AECP (25 and 50 mg/kg p.o.) showed significant (P < 0.05) decrease in serum levels of ALT and AST compared to the paracetamol treated group on day 10 of study [Figure 2].

**Wound Healing Property Incision model:** Animals treated with DXM (0.34 mg/kg i.m. on first day and 0.17 mg/kg i.m. on alternative days for 7 days) and AECP (25 and 50 mg/kg p.o.) showed significant (P < 0.05) increase in tensile breaking strength of sutured skin compared to control group [Table 2].

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Hb (g/dL) Day 1</th>
<th>Hb (g/dL) Day 5</th>
<th>Hb (g/dL) Day 10</th>
<th>Serum total bilirubin (mg/dL) Day 1</th>
<th>Serum total bilirubin (mg/dL) Day 5</th>
<th>Serum total bilirubin (mg/dL) Day 10</th>
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<tr>
<td>D.W. (5 mL/kg)</td>
<td>10.8±0.52</td>
<td>10.83±0.57</td>
<td>10.4±0.43</td>
<td>1.08±0.08</td>
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<td>PHZ (5)</td>
<td>11.1±0.65</td>
<td>6.3±0.4</td>
<td>6.56±0.26</td>
<td>0.95±0.02</td>
<td>5.13±0.13@</td>
<td>4.25±0.28$#</td>
</tr>
<tr>
<td>Silymarin (100)</td>
<td>11.97±0.21</td>
<td>7.03±0.06</td>
<td>7.33±0.23$*</td>
<td>1.10±0.08</td>
<td>4.99±0.04@</td>
<td>1.41±0.16$</td>
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<tr>
<td>AETT (25)</td>
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<td>8.03±0.33</td>
<td>8.2±0.05$*</td>
<td>1.0±0.05</td>
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<td>1.08±0.09$</td>
</tr>
<tr>
<td>AETT (50)</td>
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<td>7.86±0.75</td>
<td>8.46±0.23$*</td>
<td>1.02±0.07</td>
<td>5.49±0.29@</td>
<td>0.99±0.45</td>
</tr>
</tbody>
</table>

*Fig. 1. Effect of the aqueous extract
*Fig. 2. Effect of the aqueous extract

**Excision model:** Percentage wound healing and epithelization days: Animals treated with DXM (0.34 mg/kg i.m. on 1st day and 0.17 mg/kg i.m. on alternative days till epithelization) and AECP (25 and 50 mg/kg p.o.) showed significant (P < 0.05) increase in wound contractions [Figure 2] and decrease in epithelization period as compared to control group [Table 2].
In order to establish a scientific basis for utilization of Terminalia tomentosa in the treatment of hyperbilirubinemia, it was decided to evaluate the bilirubin lowering activity in PHZ and paracetamol-induced hyperbilirubinemia rats. Earlier reports revealed that PHZ induced hyperbilirubinemia rats showed marked increase in serum total bilirubin bark the reason for which would be excess hemolysis of the RBC’s leading to over production of the bilirubin causing hyperbilirubinemia. Paracetamol treated rats showed marked increase in serum total bilirubin, ALT and AST bark the mechanism of which is acute hepatocyte necrosis due to formation of N-acetyl-p-benzoquinoneimine (NAPQI) and saturation of sulfate and glucuronide pathways of paracetamol metabolism. Silymarin a unique flavonoid complex is a substance with documented hepato protective property by its cell membrane stabilizing property. Similarly, the number of flavonoid components, flavono lignans were found to be present in Terminalia tomentosa Hence, the bilirubin lowering activity of AETT was studied along with Silymarin and comparing both with jaundiced groups.In the present study, results of both hyperbilirubinemic model viz., PHZ and paracetamol, revealed a significant (P < 0.05) decrease in the serum total Terminalia tomentosa barks in PHZ and paracetamol treated animals with increase in Hb in PHZ treated animals and decrease in Serum ALT and AST levels when compared with vehicle-treated group and also results were observed to be more with vehicle-treated and group also results were observed to be similar to that of Silymarin treated groups. Dexamethasone is a glucocorticoid possess, a marked anti-inflammatory property along with its capacity to stimulate connective tissue growth factor (CTGF) expressions in normal tissues and organs causing fibroblast proliferation and extracellular matrix deposition which may serve as a basis for its use as wound healing agent for a preclinical study. Wound healing property of T. TOMENTOSA was studied using two different models viz., incision and excision wound model. The results of incision wound showed a significant increase in breaking strength of sutured skin. In excision study, the animals treated with AETT showed a significant increase in wound contraction, increased percentage wound healing with a decrease in epithelization period. This result was in agreement with that of a previous study by Shilpa et al. who reported that treatment with TERMINALIA TOMENTOSA possess potent wound healing activity in excision and incision wound model.

Thus, this paper presented the Immunomodulatory and wound healing activity of aqueous extract of Terminalia tomentosa barks.

REFERENCES