EVALUATION OF HYPOGLYCEMIC AND WOUND HEALING ACTIVITIES OF LANTANA WIGHTIANA WALL. EX GAMBLE LEAVES

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ABSTRACT
Lantana wightiana Wall, a member of Verbenaceae is an unarmed sub shrub, up to 3 m tall. The present study was aimed at evaluating the hypoglycemic and wound healing activities of Lantana wightiana. The leaves were collected, shade dried and extracted with water and ethanol-water (1:1) by maceration for 72h. The aqueous extract was used for anti diabetic activity in both normal and alloxan induced diabetic rats and hydro alcoholic extract along with leaf juice were evaluated for their wound healing activity in Excision wound model. Studies on the aqueous leaf extract of the plant L. wightiana revealed that the extract caused significant reduction in the blood glucose levels in the rats. The extract was found to produce marked reduction in blood glucose concentration between 2-4 hours of administration in alloxan induced hyperglycemic rats at tested dose levels. The studies on wound healing activity revealed that the nitrofurazone treated animals showed 95.19% healing on 14th day of study. On the other hand, the hydro alcoholic extract and leaf juice treated group showed 87.13% and 94.19% healing respectively.

Keywords: Lantana wightiana, Hydro alcoholic extract, Alloxan, Hypoglycemic activity, wound healing activity.

INTRODUCTION
Lantana wightiana Wall, a member of Verbenaceae is an unarmed sub shrub, up to 3 m tall; tender parts scabrous. Leaves are elliptic-ovate, base rounded to acute, apex sub acute, softly appressed-pubescent with 2-4 x 1.5-3 cm long. Flowers white in elongated spikes. Berries are brown-black when ripe1. The plant is occasional in the scrub jungles of Guntur and widely distributed in Samarilakota (Eastgodavari), Gopanapalem (West Godavari), Bollthalami (Nalgonda), and Pakala (Warangal) of Andhra Pradesh, India2-3. The plant was reported for insecticidal activity4. Lantana wightiana is one of the plants used in the treatment of various ailments, there was no significant scientific data carried out on this plant. Hence, the present study was under taken to evaluate the hypoglycemic and wound healing activities of the plant.

MATERIALS AND METHODS

Plant material
Fresh leaves about 2 kg of Lantana wightiana were collected during early summer, 2011 from young matured plants from the rural belt of Pakala, Warangal and authenticated by Prof. V. S. Raju, Taxonomist, Kakatiya University, Warangal, Andhra Pradesh, India. A voucher specimen (KSR/13/2011) was deposited in the Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India. The leaves were dried under shade, pulverized, passed through sieve no. 40 and used for further studies.

Preparation of extract
The powdered leaves (250 g) of L. wightiana were extracted separately with 2 liter of distilled water by decoction for 10 min and with 2 liter of ethanol-water (1:1) by maceration for 72h. The liquid extracts were concentrated under vacuum to yield dry extracts [aqueous extract, (AELW) yield 18.26% w/w and hydro alcoholic extract (HALW) 11.36% w/w with respect to dry material]. The aqueous extract was suspended in 0.5% w/v sodium carboxy methyl cellulose in distilled water and used for the anti diabetic screening. The hydro alcoholic extract incorporated in simple ointment I.P. (10% w/w) and fresh leaf juice (JJW) (obtained by expression of 100 g of fresh leaves) was used for the wound healing activity5, 6.

Experimental animals
Adult Wistar rats (150-200g) and Swiss albino mice (for toxicity studies) of either sex were used in the studies. The animals were kept in standard polypropylene cages at room temperature of 30 ± 2 °C and 60-65 % relative humidity. All the experimental procedures were approved by Institutional animal ethical committee of Vaagdevi College of Pharmacy, Hanamkonda, Andhra Pradesh, India vide approval No. 1047/AC/09/PCPSEA.

Acute toxicity studies
The aqueous extract of leaves of L. wightiana was screened for the gross behavioral and toxicity studies in selected Swiss albino mice. Groups of mice comprising six animals each were treated with 100, 200, 400,800, 1000, 2000, 3000 and 4000 mg/kg of the extract suspended in 0.5% w/v sodium carboxy methyl cellulose were administered orally, via a gastric catheter. The animals were then observed continuously for first four hours for any behavioral changes and for mortality if any at the end of 72 h. However, no mortality was observed in the animals. Hence, AELW at a dose of 200 and 400mg/kg were selected for the present study.

Anti diabetic evaluation of L. wightiana
The anti diabetic screening of the aqueous extract of the leaves of L. wightiana was studied on both alloxan induced diabetic rats and normoglycemic rats.

Anti diabetic evaluation in hyperglycaemic rats
The acclimatized animals were kept fasting for 24 hours and injected intraperitoneally a dose of 120 mg/kg of alloxan monohydrate in normal saline. After one hour, the animals were provided feed ad libitum. The blood glucose level was checked before alloxanisation and 24h after alloxanisation by withdrawing blood from the tip of the ear of each rat under mild ether anaesthesia. The blood glucose level was measured with haemoglucostrips supplied by M/s Pulsatum Health Care Pvt. Ltd., Bangalore, India with the help of a Pulsatum blood glucose monitor. Animals were considered diabetic when the blood glucose level was raised beyond 200mg/100ml of blood. This condition was observed at the end of 48h after alloxanisation. The animals were segregated into four groups of six rats in each. Group-I served as control and received vehicle (2ml/kg) through oral route. Group-II received glibenclamide (2.5mg/kg).
Group-III and IV received the aqueous extract at doses of 200 and 400 mg/kg in a similar manner. Blood samples were collected from each rat by cutting the tip. Blood glucose level was estimated at 0 h, 1 h, 2 h, 4 h and 8 h respectively. The results were given in Table-1.

**Anti diabetic evaluation in normoglycaemic rats**

The animals were fasted for 18 hours, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the tail of each rat and the blood glucose was estimated as above. The normal rats were then divided into four groups of six animals each. Group-I served as control and received vehicle (2ml/kg) through oral route. Group-II received glibenclamide (2.5mg/kg). Group-III and IV received the aqueous extract at doses of 200 and 400 mg/kg in a similar manner. Blood glucose levels were monitored after 1, 2, 4 and 8 h of administration of single dose of test samples. The results were presented in Table-2.

**Wound healing activity of L. wightiana**

For testing the wound healing property, a method known as Excision wound model was selected. The animals were divided into four groups of six in each. The skin hair was removed by using a depilatory cream. Light incisions were made on the cleared surface by cutting the skin of the animals under mild ether anaesthesia. The area of the wound was measured (sq. mm) immediately by placing a transparent polythene graph paper over the wound and then traced the area of the wound on it. This was taken as the initial wound area reading. All the test samples were applied topically. Group-I served as control. Group-II served as reference to which nitrofurazone (0.2% w/w in simple ointment) was applied topically. Group-III animals were treated with the hydro alcoholic extract (10% w/w in simple ointment) and the Group-IV animals with the juice of the fresh leaves in a similar manner. All the test samples were applied twice daily. The wound area of each animal was measured on 1st, 4th, 8th, 11th and 14th day. The percentage healing was calculated from the days of measurements of wound area. The results were depicted in Table-3.

**Statistical Analysis**

The Mean ± S.E.M. Significance of differences between control and treated groups was determined using Student’s t-test and the level of significance was set accordingly.

**RESULTS AND DISCUSSION**

The purpose of choosing alloxan monohydrate as the diabetes-inducing agent was that it is known to produce diabetes mellitus irreversibly with a single dose administration by selective necrotic action on the beta cells of pancreas leading to insulin deficiency. Insulin deficiency leads to various metabolic aberrations in animals viz., increased blood glucose level, decreased protein content, increased levels of cholesterol and triglyceride. It is well known that the level of glycemic control is the major determinant of serum level of triglyceride. Several investigators demonstrated that near normalization of the blood glucose level resulted in significant reductions in levels of plasma cholesterol, and triglyceride level. The studies on the aqueous leaf extract of the plant *L. wightiana* revealed that the extract caused significant reduction in the blood glucose levels in the rats. The extract was found to produce marked reduction in blood glucose concentration between 2-4 hours of administration in alloxan induced hyper glycaemic rats at tested dose levels (fig. no. 2).

However, in normoglycaemic animals, the extract at 400mg/kg dose level produced marked reduction of blood glucose between 2-4 hours of administration (fig. no. 1). When compared with the standard drug glibenclamide, the extract caused noticeable reduction in the blood glucose level in both alloxanised hyper glycaemic and normoglycaemic rats. Except that the onset of action of glibenclamide was noticed from the first one hour while that of the extract was from the 2nd hour at a dose level of 400mg/kg.

The repair of wounds involves different phases including contraction, the formation of epithelialisation and fibrosis. The biological response regulating the body’s own cellular defense mechanisms contributes to the wound and its repair. The studies on wound healing activity (fig. no. 3) revealed that the nitrofurazone treated animals showed 95.19% healing on 14th day of study. On the other hand, the extract treated group showed 87.13% healing and the leaf juice treated groups exhibited 94.19% wound healing.

**CONCLUSION**

The comparable effect of the *Lantana wightiana* extract with glibenclamide may suggest similar mode of action, since alloxan permanently destroys the pancreatic β-cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extract possesses extra pancreatic effects. The extract and leaf juice were also shown significant wound healing activity. So further study is needed to isolate and characterize the phytoconstituents responsible for the said activities.

**REFERENCES**

TABLE-1: EFFECT OF AQUEOUS LEAF EXTRACT OF L. WIGHTIANA ON THE BLOOD GLUCOSE CONCENTRATION IN ALLOXAN INDUCED HYPERGLYCAEMIC RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose conc. (mg/dl.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>I</td>
<td>0.5% w/v Sodium CMC 2 ml/kg</td>
<td>265.6±11.82</td>
</tr>
<tr>
<td>II</td>
<td>Glibenclamide 2.5 mg/kg</td>
<td>288.6±22.12</td>
</tr>
<tr>
<td>III</td>
<td>AELW 200 mg/kg</td>
<td>257.1±13.72</td>
</tr>
<tr>
<td>IV</td>
<td>AELW 400 mg/kg</td>
<td>277.8±18.16</td>
</tr>
</tbody>
</table>

Results expressed as Mean ± S.E.M from six observations. * P < 0.01; ** P < 0.001

TABLE-2: EFFECT OF AQUEOUS LEAF EXTRACT OF L. WIGHTIANA ON THE BLOOD GLUCOSE CONCENTRATION IN NORMOGLYCAEMIC RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose conc. (mg/dl.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>I</td>
<td>0.5% w/v Sodium CMC (Vehicle)</td>
<td>96.16±3.12</td>
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<tr>
<td>II</td>
<td>Glibenclamide (2.5 mg/kg)</td>
<td>98.56±3.5</td>
</tr>
<tr>
<td>III</td>
<td>AELW (200 mg/kg)</td>
<td>92.14±3.7</td>
</tr>
<tr>
<td>IV</td>
<td>AELW (400 mg/kg)</td>
<td>88.46±3.4</td>
</tr>
</tbody>
</table>

Results expressed as Mean ± S.E.M from six observations. * P < 0.01; ** P < 0.001

TABLE-3: WOUND HEALING ACTIVITY OF THE LEAF HYDRO ALCOHOLIC EXTRACT AND FRESH LEAF JUICE OF L. WIGHTIANA IN EXCISED RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Percentage inhibition of wound on the day of study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Nitrofurazone (0.2% w/w)</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>HALW (10% w/w)</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>LJLW</td>
<td>0</td>
</tr>
</tbody>
</table>

Results expressed as Mean ± SEM from six observations. * P < 0.001 on 14th day of study.

Fig. No. 1 Effect of aqueous leaf extract of L.wightiana on the blood glucose concentration in alloxan induced hyperglycaemic rats.
Fig. No. 2 Effect of aqueous leaf extract of *L. wightiana* on the blood glucose concentration in normoglycaemic rats.

Fig. No. 3 Wound healing activity of the leaf hydro alcoholic extract and fresh leaf juice of *L. wightiana* in excised rats.

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