Materials
and
Methods
MATERIALS AND METHODS

(A) TOOLS

1. Plant Material:

The leaves of *Vitex negundo* and *Ficus religiosa* were collected around Jhansi city, (Chandpura & Bangawa forests) in 2001 from the wild. A voucher specimen of Vitex negundo and Ficus religiosa (authenticised by Dr.C.K.Rai of Ayurvedic Medical College, Jhansi) has been deposited at Institute of Pharmacy, Bundelkhand University, Jhansi (U.P.), India.

The leaves were dried under shade and after it, powdered to a fine texture and 100g each of the dried leave was extracted with water and alcohol (absolute). The extracts were concentrated under vaccum and the residues were used in the experiments. The dried leave extracts were freshly suspended in normal saline just before administration (using 1% acacia) by oral route.

2. Animals:

The studies were conducted on albino rats (weighing 150-250g) and mice (weighing 18-22g): approved by CPCSEA, Chennai, India (registration number 716/02/a/ CPCSEA). The test animals were allowed to acclimatise to animal house conditions for two weeks prior to starting of the experiments. The animals were fed standard rodent pellet diet and tap water was allowed *ad libitum*. Each experimental group consisted of 6 animals housed in separate cages.

3. Chemicals:

All the chemicals/solvents to be used in the study were AR grade
4. Statistical Analysis:

All the grouped data were statistically evaluated for the mean ± S.E. (standard error of the mean). The significance of various treatments and evaluation of data were calculated using student's unpaired t-test suitable method; & P<0.05 was accepted as significant.

(B) METHODOLOGY

(a) Acute Toxicity Determination:

The acute toxicity (LD$_{50}$) profile of the extract were evaluated in swiss albino mice according to the method of Lorke (1983) with some modification, briefly, (This method was carried out in two steps; the initial investigation in which nine animals were used, three animals per treatment group and widely differing dose ranges, 1:10:100 mg/kg respectively of the extract per body weight were administered and were observed for 24 h. Based on the results of the first step, the second step was initiated in which specific dose ranges of the extract were administered to four treatment groups of ten animals each. The LD$_{50}$ was then calculated based on the pattern of death observed in the second step.

(b) Chronic Toxicity Study:

Adults healthy albino rats (Charles Foster strain) of either sex weight 150-250g were used in the present study, these animals were kept in polypropylene cages (six rats per cage) under identical animal house conditions and provided with pelleted 'Gold Mohaur' rat feed (manufactured by Hindustan Lever Limited ; India) and water were given ad libitum. The rats were equally divided into the following groups:

Group I - (control the rats of this group were fed standard rat feed of normal saline 5ml/kg, po).
Group II – Diabetic rats treated with standard Glibenclamide 500μg/kg, po.

Group III – Alcoholic extract of Vitex negundo treated with 150mg/kg, po dose.

Group IV – Alcoholic extract of Vitex negundo treated with 300mg/kg, po dose.

Group V – Alcoholic extract of Vitex negundo treated with 600 mg/kg, po dose.

Group VI – Water extract of Vitex negundo treated with 150 mg/kg, po dose.

Group VII – Water extract of Vitex negundo treated with 300 mg/kg, po dose.

Group VIII – Water extract of Vitex negundo treated with 600 mg/kg, po dose.

Group IX – Water extract of Ficus religiosa treated with 150 mg/kg, po dose.

Group X – Water extract of Ficus religiosa treated with 300 mg/kg, po dose.

Group XI – Water extract of Ficus religiosa treated with 600 mg/kg, po dose.

The animals were treated orally, daily for 30 days continuously. The treated animals were monitored daily for any visible signs of toxicity including any behavioral alterations. At the end of the treatment period, the animals were sacrificed and vital organs like kidney, liver, heart and pancreas were excised and observed for gross and histopathological signs of toxicity.
Antidiabetic Evaluation

Diabetes was induced in rats by oral and parenteral route by Alloxan (150mg/kg, i.p.) and Streptozotocin (50mg/kg, i.p.)

The antidiabetic model of Anuradha and Ravikumar (2001), was adapted for this study with some modifications. Male wistar rats weighing 150-250 g were used for the study. The rats were maintained on standard rat feed with water ad libitum. The non-diabetic animals were selected for the study, the baseline plasma glucose level were determined prior to selection of the animals. All the animals were given 150 mg/kg body weight, freshly prepared alloxan monohydrate in saline and 50mg/kg STZ in saline; by i.p. route. The alloxan and STZ - induced diabetic rats were divided into five-groups of six each.

Experimental Groups

Group -I: Normal rats treated with physiological saline (5ml/kg, po) was considered as the control group

Group -II: Diabetic rats induced by alloxan (150mg/kg, i.p.).

Group -III: Diabetic rats induced by streptozotocin (50mg/kg, i.p.).

Group - IV: Diabetic rats treated with Glibenclamide (500μg/kg, po.) was considered as standard for coming to conclusion.

Group-V: Diabetic rats treated with various plant extracts (Alcoholic and water extracts of Vitex negundo and Ficus religiosa) of various doses (150, 300 and 600 mg/kg, po.).

General Procedure
The rats with a fasting blood glucose level higher than 200 mg/dl were included in the study. At the end of 30 days, the rats were fasted overnight
and killed by cervical dislocation. The throat was cut opened. 0.5 ml of blood were dropped on the reagent pad of the one touch strip. The strip was inserted into glucometer (Johnson & Johnson) and the reading was noted.

(d) **Histopathology**

At the end of 30 days of treatment, the animals were autopsied & the vital organ, viz. Liver, Kidney, Heart and Pancreas were excised, these organs were fixed in buffered 10% formalin & 3μm thick paraffin sections were stained with haematoxylin & eosin according to Lillie, et al (1965) and microscopic examinations were carried out by Electron Microscope using Nikon – FX – 35 DX camera (Japan).

(e) **Haematology**

Blood samples collected out of 6 rats from each group were examined for the peripheral count of Red Blood Cells (RBCs), total White Blood Cells (WBCs) & differential count of WBCs were carried out according to the method of Bharucha et al (1976).