ANTIIMPLANTATION ACTIVITY OF THE METHANOLIC EXTRACT OF CORDIA DICHOTOMA LAM. BARK IN RATS

Parimal P. Katolkar*1, Bhumesh. E. Wanjari1, Tul sidas. P. Nim bekar1, Nandkishor. J.Duragkar2

1Manoharbhai Patel Institute of Pharmacy (B. Pharm.), Kudwa, Gondia
2Sharad Pawar College of Pharmacy, Wanadongri, Nagpur-441110, India.

E-mail of Corresponding Author: p_k_7995@yahoo.co.in

Abstract
In the present study, antiimplantation activity of methanolic extract of Cordia dichotoma Lam. bark was evaluated. The methanolic extract of bark of Cordia dichotoma showed significant antiimplantation activity. Pretreatment with methanolic extract showed significant inhibition of number of implant site at a dose of 100-mg/kg-body weight. There was no change in ovulation; hence the antiimplantation activity observed in the present study with Cordia dichotoma bark can be attributed largely to its inhibition of number of implants. However, an investigation of the antiimplantation activity showed no increase in body weight or uterine content of blood glucose, cholesterol and triglyceride levels when compared with control group.

Keywords: Cordia dichotoma, antiimplantation activity, antifertility activity, methanolic extract.

1. Introduction
Cordia dichotoma L. (Boraginaceae) is tree of tropical and subtropical regions, commonly known as Lasaura/Lasura. It is a medium sized tree with short crooked trunk, leaves simple, entire and slightly dentate, elliptical-ovate to broad ovate with round and cordate base, flower white, fruit drupe, yellowish brown, pink or nearly black when ripe with viscid sweetish transparent pulp surrounding a central stony part.1 It grows in sub-Himalayan tract and outer ranges, ascending up to about 500 m elevation.2 It is used as immunomodulator, antidiabetic, anthelminitic, diuretic and hepatoprotective in folklore medicine. Cordia dichotoma seeds has disclosed the presence of α-amyrins, betulin, octacosanol, lupeol-3-rhamnoside, β-sitosterol, β-sitosterol-3-glucoside, hentricontanol, hentricontane, taxifolin-3, 5-dirhamnoside and hesperitin-7-rhamnoside. The seed contain α- amyrin and taxifolin 3, 5-dirhamnoside, which shows significant anti-inflammatory activity by an oral dose of 1gm/kg in albino rats. The seeds of this plant reported to contain fatty acids and flavonoids.3 The goal of the present study was to investigate the antiimplantation activity of methanolic extract of Cordia dichotoma bark, also attempt to investigate some biochemical parameters, mainly blood glucose, cholesterol and triglycerides.

2. Materials and Methods
2.1 Plant materials: Cordia dichotoma Lam. Bark was collected in month of August 2011 from local Region of Gondia District. It was authenticated at Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur (Voucher No. 9087). The Cordia dichotoma bark were air dried in shade, under normal environmental conditions and then subjected to size reduction to get coarsed powder. The coarsed powder material was charged into the Soxhlet apparatus for defatting with petroleum ether (60-80) followed by extraction with methanol. The methanolic extract were subjected to preliminary phytochemical screening.4

2.2 Animals: Wistar strain rats of either sex weighing between 200-250 g procured from Shree Farms, Bhandara were used for the present investigation. Animal Ethical Committee had approved the experimental protocol under the guidelines of CPCSEA, New Delhi (No. 928/ab/06/CPCSEA). The rats were housed at controlled temperature (25±2°C), relative humidity (65±10%), light and dark cycle (12:12h) and fed standard pellet food, water ad libitum. The initial body weight of each animal was recorded. The vaginal smear of the rats was studied microscopically for estrus cycle every morning. Only female rats with normal estrus cycle were selected for the antiimplantation activity.
2.3 Acute Toxicity Study: Toxicity study of methanolic extract of *Cordia dichotoma* Lam. Bark was carried out in mice according to OECD guidelines. Extract at different doses up to 1000mg/kg body weight, orally was administered and animals were observed for behavioral changes, any toxicity and mortality up to 48 h. There was no toxic reaction or mortality and found to safe. Based on acute toxicity result we have selected 50 mg/kg body weight and 100-mg/kg-body weight for antiimplantation activity.

2.4 Anti-implantation activity

Proven fertile female rats of Wister strain weighing 200-250g were screened for 2-3 estrous cycles by examining the vaginal smears. The rats that showed normal cycles for two successive examinations were selected for study. The method of Khanna and Chaudhary was followed with necessary modifications; the rats in proestrous and estrous stages were caged with fertile male in the ratio 2:1. The following day vaginal smears were examined and the appearance of the sperms clusters in the smears was recorded as day 1 of pregnancy. Control animals in the Group-I received on Tween-80 (1%) + saline as vehicle only. Methanolic extracts of *Cordia dichotoma* bark at 50 mg/kg & 100mg/kg were administered orally to Group-II and Group-III in the form of suspension of dried powders with Tween-80 (1%) + saline respectively. On day 10 laprotomy was performed under light ether anesthesia to examined uteri for implant number and size. Then the abdomen was closed and rats were allowed to recover and deliver after full term pregnancy. Those rats, that not deliver, were laprotomised on the day 21 and uteri were examined for implantation sites. The fertility test was considered as positive if implantation sites were observed and negative in their absence. The born litters were observed for teratogenic abnormality, if any.

2.5 Biochemical studies: The present study besides evaluating the antiimplantation effect, also attempts to investigate some biochemical parameters, mainly blood glucose, cholesterol and triglyceride.

2.6 Statistical analysis: The values are expressed as Mean±SEM. ANOVA followed by Tukey-Kramer multiple compression test was performed to determine the difference between Mean and \(p=0.05\) considered as statistically significant.

3. Result

Preliminary phytochemical study of methanolic extract of *Cordia dichotoma* bark showed the presence of saponins, flavonoids, glycosides, alkaloids, tannins, sugar and amino acids.

After treatment with *Cordia dichotoma* bark, body weights were reduced up to 21 days. Maximum reduction was observed in 100mg/kg body weight of *Cordia dichotoma* bark i.e. 5.42 % compared to 50 mg/kg methanolic extract of *Cordia dichotoma* bark i.e.3.98 %. The rise in body weight was + 4.05 % in control group of rats (table 1). Maximum percent inhibition or reduction of implantation on born litters was observed in methanolic extract (100 mg/kg body weight) and methanolic extract (50mg/kg body weight) of *Cordia dichotoma* bark. All rats delivered in control group (table 2). Biochemical changes observed in *Cordia dichotoma* bark extracts treated rats showed depletion in blood glucose, cholesterol and triglyceride levels as compared to Group-I i.e. control group (table 3). In Group-III i.e. methanolic extract (100mg/kg) of *Cordia dichotoma* bark, percent change observed was -7.38% in blood glucose, - 24.46 % in cholesterol, and -13.73% in triglyceride. In Group-II i.e. methanolic extract (50mg/kg) of *Cordia dichotoma* bark, percent change observed was –5.79 % in blood glucose, -10.29% in cholesterol, and - 7.91% in triglyceride.

4. Discussion

In this present study methanolic extracts of *Cordia dichotoma* bark evaluated for its antiimplantation activity. In this antiimplatation model, we determined the body weight, percent pregnancy inhibition (implatation) and biochemical parameters. We found that decreases in body weight after extract treated group and also percent inhibition of pregnancy (implantation) increases as the dose of methanolic extracts of *Cordia dichotoma* bark increases.

In biochemical parameters such as blood glucose, cholesterol and triglyceride showed the decreased in levels as compared to control group. Since the cholesterol is the precursor of the steroidogenesis of ovarian endocrine tissues. These decreases in cholesterol level can cause diminution of ovarian steroidogenesis.

It is well known fact that for implantation and sustenance of pregnancy, exact equilibrium of
secretion estrogen and progesterone is necessary. Any imbalance in the level of these hormones can cause antiimplantation or induce abortion.9

Presence of chemical constituents in Cordia dichotoma bark saponins, glycosides and flavonoids these constituents might be responsible for its antiimplantation activity. The present experimental findings suggest that, methanolic extract of Cordia dichotoma bark has antiimplantation activity, hence its antiimplantation action responsible for its antifertility activity. Further detailed study using different animal species to establish its antifertility activity and also undersigned cellular mechanism of action.

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Table No. 1: Body weight changes in control as well as experimental female rats treated with methanolic extracts of Cordia dichotoma bark (MCD)

| Sr. No | Duration of treatment | Control (Wt. in gm) | MCD 50mg/kg (Wt. in gm) | MCD 100mg/kg (Wt. in gm) |
|--------|-----------------------|---------------------|------------------------|-------------------------|
| 1      | Initial weight        | 242.5±7.04          | 246.83±4.56            | 245.83±2.91             |
| 2      | 7 days                | 245.66±7.24 (+1.30%)| 244.5±4.73 (-0.94%)    | 242.16±2.82 (-1.49%)   |
| 3      | 14 days               | 250.00±6.83 (+3.09%)| 242.5±4.89 (-1.75%)    | 238±2.74 (-3.18%)      |
| 4      | 21 days               | 252.33±6.93 (+4.05%)| 237±4.59 (-3.98%)      | 232.5±2.63 (-5.42%)   |

Values are Mean ±SEM of six animals per group Test, Figure in parenthesis indicate percent change over control.

Table No. 2: Effect of methanolic extracts of Cordia dichotoma bark (MCD) on reduction in pregnancy in rats

| Gr. No. | Treatment | Dose Mg/kg (Body weight) | No. of rats pregnant/ Treated | % Reduction in pregnancy | No. of implant in individual rats | No. of rats delivered (No. of pups) |
|---------|-----------|--------------------------|------------------------------|--------------------------|-------------------------------|-----------------------------------|
| I       | Control   | Tween-80+ Saline        | 6/6                          | 0                        | 13,12,9,7,8,10                | 6(13,12,9,7,8,10)                   |
| II      | MCD 50    | 50                       | 3/6                          | 50                       | 3,2,0,0,1,0                  | 3(3,2,0,0,1,0)                      |
| II      | MCD 100   | 100                      | 0/6                          | 100                      | 0,0,0,0,0,0                  | 0(0,0,0,0,0,0)                      |

Table No. 3: Effect of methanolic extracts of Cordia dichotoma bark on blood glucose, cholesterol and triglycerites.

| Sr. No | Biochemical parameters (mg/100ml) | Control | MCD 50mg/kg | MCD 100mg/kg |
|--------|----------------------------------|---------|-------------|--------------|
| 1      | Blood glucose 77.31±1.04         | 72.83±0.31** (-5.79%) | 71.6±0.53*** (-7.38%) |
| 2      | Cholesterol 70.61±0.19           | 63.34±0.71***(-10.29%) | 53.34±0.41***(-24.46%) |
| 3      | Triglyceride 156.78±1.06         | 144.37±2.15***(-7.91%) | 135.25±0.62***(-13.73%) |

*p<0.05, ***p<0.001. **p<0.01 when compared with control group.