DISCUSSION
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The indiscriminate and uncontrolled growth of human population in our country is one of the biggest problems we are facing today. Over the ages, plant preparations have been used effectively by Practitioners of traditional medicine to control birth. Long historical use of many practices of traditional medicine, including experience passed on from generation to generation has demonstrated the safety & efficacy of traditional medicine. However, scientific research is needed to provide additional evidence on their safety and efficacy. The present study was an attempt to substantiate the folklore claims using animal models and to generate preclinical data on the safety and efficacy of leaves of three selected plants used traditionally as antifertility agents by tribal of Orissa. The three plants selected were Zizyphus jujuba Mill& Lamk (Family: Rhamnaceae), Stephania hermandifolia Walp (Family: Menispermaceae), Cissampelos pareira Linn (Family: Menispermaceae).

The petroleum ether, hexane, chloroform, acetone, ethanol, methanol and aqueous extracts of Zizyphus jujuba, Stephania hermandifolia and Cissampelos pareira were studied for their antifertility activity in female albino rats. In the control group receiving gum acacia 0.1% w/v from day 1 to 7 of pregnancy, all rats showed implantation sites and all delivered litters. The petroleum ether, hexane and acetone extracts of Zizyphus jujuba at the dose of 100, 200, 400 mg/kg did not show any reduction in pregnancy. All rats showed implantation sites and all delivered litters. The chloroform extract of Zizyphus jujuba at the dose of 100 and 200 mg/kg did not show any reduction in
pregnancy. However at a dose of 400 mg/kg showed 16.66% reduction in pregnancy (Table 9). The methanolic extract of *Zizyphus jujuba* at the dose of 100, 200, 400 mg/kg showed 33.33%, 50% and 66.66% reduction in pregnancy respectively (Table 12). The aqueous extract of *Zizyphus jujuba* at the dose of 100, 200, 400 mg/kg showed 16.66%, 33.33% and 50% reduction in pregnancy respectively (Table 13). The ethanolic extract of *Zizyphus jujuba* at the dose of 100, 200, 400 mg/kg showed 33.33%, 50% and 83.33% reduction in pregnancy respectively (Table 11). This revealed that the ethanolic extract of *Zizyphus jujuba* was found to be the most effective amongst the seven extracts. The extract showed dose dependent reversible anti fertility effect. Further, it revealed that the ethyl acetate fraction of *Zizyphus jujuba* was found to be the most active fraction of the ethanolic extract as indicated in the Table 14.

In case of *Stephania hermandifolia*, its petroleum ether, hexane and acetone extracts at the dose of 100, 200, 400 mg/kg did not show any reduction in pregnancy. All rats showed implantation sites and all delivered litters. The chloroform extract at the dose of 100, 200, 400 mg/kg showed 50%, 66.66% and 100% reduction in pregnancy respectively (Table 18). The ethanolic extract at the dose of 100, 200, 400 mg/kg showed 16.66%, 33.33% and 50% reduction in pregnancy respectively (Table 20). The methanolic extract at the dose of 100, 200, 400 mg/kg showed 33.33%, 50% and 66.66% reduction in pregnancy respectively (Table 21). The aqueous extract at the dose of 100, 200, 400 mg/kg showed 66.66%, 83.33% and 100% reduction in pregnancy respectively (Table 22). So the aqueous and chloroform extracts of *Stephania hermandifolia* were found to be the most active. This is in confirmation of the earlier reports of...
reproductive effects of ethnomedicinal formulation of tape-vine leaves in female rats.\textsuperscript{11} The aqueous extract of leaves of \textit{Stephania hernandifolia} inhibited ovarian gametogenesis.\textsuperscript{53} Since already some work has been done on the aqueous extract of \textit{Stephania hernandifolia}, the chloroform extract was taken for study in the present investigation.

\textit{Cissampelos pareira} though belongs to the same family as that of \textit{Stephania hernandifolia} did not show any antifertility effect. So the tribal people might be using \textit{Cissampelos pareira} instead of \textit{Stephania hernandifolia} either in ignorance or due to confusion as both plants look similar.

There was no post implantation loss of conceptus in any of the treatments as number of pups delivered was found to be equal to number of implantation sites in individual rat. There was no evidence of teratogenicity by the administration of any of the extracts of the three selected plants.

Reversibility after stopping the treatment is one of the major criteria of a good antifertility agent. The ethanolic extract of \textit{Zizyphus jujuba} and its ethyl acetate fraction showed reversible antifertility action since complete recovery of fertility was observed following withdrawal of the drug. The chloroform extract of \textit{Stephania hernandifolia} also shows reversible antifertility action.

The sequence of events in rhodents after mating consists of following stages.\textsuperscript{34}

<table>
<thead>
<tr>
<th>Stage</th>
<th>2 cell stage</th>
<th>4 cell stage</th>
<th>8 cell stage</th>
<th>Blastocyst</th>
<th>Implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>2 and 3</td>
<td>Late 3</td>
<td>Late 4</td>
<td>5</td>
<td>Late 5</td>
</tr>
</tbody>
</table>

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Thus zygote is formed during first 3 days of pregnancy, blastocyst formation takes place within 5 days and by the end of 5th day of pregnancy implantation is completed. Accordingly to detect the exact mode of anti-fertility action i.e. anti-zygotic, blastocytotoxic, or anti-implantation, the rats were given the test drugs for different periods of pregnancy. The results revealed that the mechanism of anti-fertility activity of the ethanolic extract of *Zizyphus jujuba* and its ethyl-acetate fraction might be anti-zygotic or early abortifacient action, as maximum reduction in pregnancy (83.33%) was observed when the extract as such or the fraction of the extract was administered for first three days of pregnancy, while administration for subsequent days of pregnancy failed to produce significant reduction in the pregnancy (Table 15). Similarly, the chloroform extract of *Stephania hemandifolia* showed maximum reduction in pregnancy (83.33%) when administered from day 6 to day 7 of pregnancy whereas the pregnancy was reduced by only 50% and 16.66% when administered from day 4-5 and 1-3 of pregnancy respectively (Table 23). So the mechanism of antifertility effect of *Stephania hemandifolia* might be of antiimplantation activity.

The changes in the vagina of the normal animals are believed to be due to the fluctuations and interconversions of female sex hormones, estrogen and progesterone, mainly synthesized in the ovary. The level of these hormones, however, is controlled by the pituitary gonadotrophins and hypothalamic releasing hormones. A feedback mechanism also operates whereby the pituitary releases the gonadotrophins which are in turn controlled by these estrogens and progesterone. The cornification in the vagina is mainly due to the level of stimulation of estrogen which acts directly on the vaginal epithelium.30
Reproductive cycle in mammals commences with the onset of puberty and in laboratory animals like rats. It is usually judged with the help of vaginal opening at about 38 days of age. Reproductive and general metabolic effects in mature and immature rats are manipulated with the ingestion of phytoestrogenic substances and produce effects similar to that of gonadal steroid 17-2-estradiol. Numbers of plant extracts are known to exhibit estrogenic activity in rats. A typical estrogenic compound possesses ability to increase the uterine weight but a frank estrogenic compound is that which induces cornification and opening of vagina in immature rats.\textsuperscript{120-122}

The chloroform extract of \textit{Stephania hernandifolia} caused opening and cornification of vagina in immature rats. There was also a significant increase in uterine weight in the chloroform extract treated animals (Table 24). So the chloroform extract of \textit{Stephania hernandifolia} possesses frank estrogenic activity like diethyl stilbestrol. It appears that anti-implantation effect of chloroform extract of \textit{Stephania hernandifolia} is mediated through this activity as estrogens are known to increase uterine contractility to expel the fertilized eggs. The ethanolic extract of \textit{Zizyphus jujuba} did not show estrogenic activity.

Drugs affecting uterine contractility are especially useful in the practice of obstetrics and gynecology. Drugs that increase uterine contractions are called oxytocics. They are used to augment and induce labour. It has long been recognized that these drugs when administered during pregnancy may adversely influence the continued development of foetus and induce expulsion of uterine contents. Abortion by ingestion of rye contaminated with ergot and the high incidence of miscarriages in women who worked in the lead industry or lead mining are classical examples.\textsuperscript{123}
The ethanolic extract of *Zizyphus jujuba* showed dose dependent in-vitro uterine contractions (Fig 23). So this also supports the abortifacient activity of *Zizyphus jujuba*.

In the phytochemical screening of *Zizyphus jujuba*, it is found to contain carbohydrates, phytosterols, saponins, phenolic compounds and tannins, alkaloids, flavonoids and volatile oil. In the phytochemical screening of *Stephania hemandifolia*, it is found to contain carbohydrates, proteins, alkaloids, and phytosterols. According to reports and literatures alkaloids, steroids, terpenoids and flavonoids do possess artifertility effect.\(^8\) The phytochemical results also indicate the presence of such compounds in the plant extracts.

Compound PKS-3 was obtained from *Zizyphus jujuba* as amorphous powder from the methanol fraction of successive ethanolic extract. Its IR spectrum exhibited important absorption bands for hydroxyl group (3391 cm\(^{-1}\)), carboxylic group (2859, 1705 cm\(^{-1}\)) and unsaturation (1640 cm\(^{-1}\)). Its mass spectrum displayed a molecular ion peak at m/z 456 corresponding to the molecular formula C\(_{30}\) H\(_{48}\) O\(_{3}\) of a triterpenoid. It showed presence of seven double-bond equivalents, five of which were adjusted in a pentacyclic carbon framework of terpenoid moiety, and one each in carboxylic group and vinylic function. The \(^1\)H NMR spectrum displayed two one proton broad signals at \(\delta\) 4.69 and 4.56 attributable to exocyclic vinylic protons H\(_{29a}\) and H\(_{29b}\). A double doublet, integrating for one proton, at \(\delta\) 3.73 was ascribed to H-3 carbinol proton that was placed in 2-orientation on the basis of its coupling constant (\(\tilde{J}\) = 5.5 and 9.5 Hz). A three proton broad signal at \(\delta\) 1.63 was assigned to methyl protons Me-30 adjacent to vinylic bond. The remaining methyl groups appeared as five broad singlets, integrating for three protons each; at \(\delta\) 1.23,
1.20, 0.91, 0.84 and 0.82 that were assigned correspondingly to Me-23, Me-25, Me-24, Me-26 and Me-27 methyl protons. On the basis of above observations, the structure of PKS-3 was elucidated as Lup-20 (29)-en-32-ol-28-oic acid. This is the first report of lupenoic acid derivative from this plant. The methanol fraction of ethanolic extract of *Zizyphus jujuba* possesses only 16.66% antifertility effect. So the triterpenoids might have a role in the abortifacient action of ethanolic extract of *Zizyphus jujuba*.

Compound PKS-1 was obtained as colourless crystals from acetone extract of *Stephania hernandifolia*. Its IR spectrum showed absorption bands at 3425 and 3311 cm\(^{-1}\) characteristic for hydroxyl group. The mass spectrum of PKS-1 showed a molecular ion peak at m/z 342 consistent with molecular formula \(\text{C}_{12}\text{H}_{22}\text{O}_{11}\) of a disaccharide. The \(^1\)H NMR spectrum displayed a two proton broad singlet at \(\delta 4.68\) assigned to H-2 and H-1' anomeric protons. Four one proton broad signals at \(\delta 4.53, 4.45, 4.32\) and \(4.31\) were ascribed correspondingly to H-5', H-2', H-4, H-4' protons of saccharide moieties. A multiplet at \(\delta 4.40\) was attributed to H-3 and H-3' protons whereas a two proton broad signals at \(\delta 3.57\) and \(3.65\) were attributed to H\(_2\)-1 and H\(_2\)-6 carbinol protons. Two doublets, integrating for one proton each, at \(\delta 3.30\) and \(3.27 (J=10.8\ Hz)\) appeared due to H\(_2\) - 6'a and H\(_2\) - 6'b carbinol protons of glucose.

On the basis of above discussion compound PKS-1 was characterized as 2-D-fructofuranosido-(5W 1'' - K-D-glucopyranoside (sucrose).

Two of the most active extracts of *Zizyphus jujuba* and *Stephania hernandifolia* were subjected to subacute toxicity study for evaluation of the safety (Table 32-36). The test extracts were administered for 90 days and after that the haematological and biochemical parameters as well as histopathology were studied.
Ethanolic extract of *Zizyphus jujuba* was found to be safer than methanolic extract. Aqueous extract of *Stephania hemandifolia* was found to be safer than chloroform extract. The chloroform extract of *Stephania hemandifolia* is found to be associated with a lot of toxicity.

A hypothetical tablet formulation of the ethanolic extract of *Zizyphus jujuba* was developed on the basis of extrapolated human dose. Three different formulations were made by using three different concentrations of binder. The formulations were optimized with respect to desired parameters. Out of the three formulations of the ethanolic extracts of *Zizyphus jujuba* the formulation F3 with highest percentage of binder (1.5% sodium alginate solution with water) was found to be the best formulation (Table 39, 40) as the hardness, disintegration time increases and friability decreases.