Reproductive Effects of Ethnomedicinal Formulation of Tape-Vine Leaves in Female Rats[1)]

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Received March 6, 2006; accepted June 2, 2006

Documented ethno-contraceptive use of Tape-vine or *Stephania japonica* (Thunb.) Miers., Syn. *Stephania hernandifolia* (Willd.) Walp. leaves is evaluated with regards to post-coital pregnancy interceptive activity of its aqueous extract (AE) and an ethnomedicinal formulation (EF) in Wistar rats. EF at 500 and 250 mg/kg doses induced 66.7% and 33.3% post-coital pregnancy interception respectively and the higher dose exhibited significant reduction in number of litters born and also anti-implantation property. In contrast, none of the dose levels of AE interfered in pregnancy but significant anti-implantation property was observed at doses of 2 and 1 g/kg, even as the higher dose produced significant reduction in number of litters born as well. EF at 500 mg/kg also exhibited significant uterotrophic activity and histological changes in uterus. Pair-wise comparison of sex hormone-levels exhibited significant increment in serum estradiol, LH and FSH but decrease in progesterone levels. Assessed blood lipid-carbohydrate profile exhibited substantial decrease in glucose, cholesterol, VLDL and triglyceride contents and significant increase in HDL. It is concluded that EF probably acts as better post-coital pregnancy interceptive agent through restriction of implantation by alteration of gonadal hormone levels and decline in blood-glucose levels that possibly disrupts oxidative energy metabolism in uterus during implantation. High surge in LH and FSH suggests negligible interference in ovulatory mechanism. This preparation also seems to be free of cardiovascular risk factors. HPTLC and HPLC analysis of both EF and AE exhibited marked chemical differences.

Key words *Stephania japonica*; *Stephania hernandifolia*; ethno-contraceptive; pregnancy interception; anti-implantation; estrogenic activity

Tape-vine or Stephania japonica (Thunb.) Miers., Syn. Stephania hernandifolia (Willd.) Walp.,[2)] (Fam. Menispermaceae) is used by ethnic tribal societies as female contraceptive.[3,4)] It is also recorded in Indian Ayurvedic system of medicine as having significant effect on the uterus.[5)] The plant is mainly distributed along India, Penang-Siam, Malay archipelago and Australia.[6,7)] Earlier it was demonstrated that a petroleum ether extract of the plant rhizomes exhibits fertility promoting activity while an alcoholic extract display contraceptive property.[8)] The alcoholic and aqueous extracts of the rhizomes were further found to possess antiimplantation property.[9)] An alkaloid, aknadine isolated from aerial parts of this plant has been reported as uterosedative.[10)] Aqueous extract of the leaves were observed to cause reduction in the activities of testicular androgenic key enzymes and plasma level of testosterone along with suppression of spermatogenesis in male rats without any hepatic and renal toxicity.[11)] Epistephanine, an another alkaloid isolated from aerial parts of this plant, produced significant adrenergic neurone blocking activity and its activity was estimated to be 1/10th of guanethidine.[12)] This alkaloid has also been reported to be potentially cytotoxic.[10)] Freeze-dried juice of the bulbs were demonstrated to have hypoglycemic effect in insulin dependent diabetes mellitus (IDDM) but marked hyperglycemic effects in non-IDDM and non-diabetic rats.[13)] The action of an alkaloidal extract of the vines on multidrug resistance reversing activity was evaluated.[14)] An alkaloid, isotrilobine was found to be as active as verapamil in reversing doxorubicin resistance in human breast cancer cells. This compound was further reported to possess antiaggregant and anti-inflammatory properties.[10)] Apart from alkaloids,[15—19)] different classes of steroids[20,21)] and phenolic compounds[20,22)] have also been reported in this species.

In contraceptive applications, Indian traditional usage documentation[3,4)] reports that 7—8 standard sized or 9—10 small fresh leaves (approx. 5 g) of the plant are usually macerated and consumed by the tribal women as suspension in water along with sugar or honey for either 5 consecutive days following onset of menstruation or after the menstruating period. This has been acknowledged to inhibit pregnancy only in that cycle. Thus, it may be hypothesized that there could be possible anti-ovulatory or anti-implantation mechanism predominant in the bioactivity. In view of this, the present work explored on comparative appraisal of post-coital pregnancy interceptive activity of an ethnomedicinal preparation (EF) and aqueous extract (AE) of leaves of the plant with an insight into the gross mechanistic aspects. Since, marketed steroidal oral contraceptives have main drawback for alteration of carbohydrate metabolism and elevated lipid profiles,[23)] so a systemic evaluation of these parameters has also been worked out in the present work.

MATERIALS AND METHODS

Materials Fresh whole plant materials in fruiting phase, collected from South-Eastern region of West Bengal state (India), were authenticated by Prof. N. D. Paria, Department of Botany, University of Calcutta and a voucher specimen was deposited. Colony-bred adult male (350—400 g), female (140—220 g) and immature female (40—60 g) Wistar rats

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maintained in air-conditioned surroundings (25±2 °C) and provided with 12 h alternate light and dark cycle for each 24 h period with regular husbandry conditions, were used for the experiments. The animals were housed in polypropylene cages with husk beds and fed on normal pellet diets (NIN) and reverse osmosis ozonized drinking water (Silicon Food & Beverages). All biological experiments were performed after obtaining official approval and as per protocol of the Institutional Animal Ethics Committee. Anaesthetic ether I. P. (TKM Pharma) was used for anesthesia prior to laparotomy. Vaginal smear examination and photomicrography were done in an AST-1 Research Binocular Microscope (BRISTOL). Progesterone, Dehydroepiandrosterone sulfate (DHEA-S), Estradiol (Equipar) and Testosterone (Merck) direct immunoenzymatic assay (EIA) systems were used for the quantitative determination of serum hormone levels. FSH and LH (Monobind) estimations were done through Chemiluminescence Immunoassay (CLIA). Lipid-carbohydrate estimations were done with standard reagent kits (Span). Sterile water for injection I. P. (Core) was used for washing antibody-coated microwells in EIA of hormones. EIA and CLIA estimations were done in Eldex 3.8 ELISA reader (Lilac) and ALFA PRIME CLI analyzer (Lilac) respectively. The lipid-carbohydrate profiles were estimated in a UV-VIS Spectrophotometer (Shimadzu UV-2550). Thin Layer Chromatography (TLC) was done in precoated silica gel $60F_{254}$ (Merck) plates (10 cm×10 cm) using Linomat 5 (Camag) automated sample application system and densitometric analysis was done in TLC scanner (Camag) at 254 and 366 nm wavelengths. HPLC analyses were performed in binary Perkin Elmer S200 model with Spheri-5 RP-8 5 μ column (4.6×250 mm) at 254 nm in UV detector (Perkin Elmer). All solvents (Merck) used were of either AR or HPLC grade.

Preparation of Test Substances The leaves were separated off from the plants and washed in distilled water. The cleaned leaves were further processed for preparation of test samples, *viz.* AE and EF. For preparation of AE, the leaf samples were shade dried for 3 months at an ambient temperature of 25—30 °C and subsequently powdered to #20—40 mesh particles. The powdered materials were extracted in distilled water (taken in the ratio 1 : 8 w/w) by refluxing for 48 h. The extract obtained was filtered and subsequently dried to a solid consistency at 70 °C. This material had an extractive value of 30.8%. The extract was administered as aqueous solutions in required dose levels. For preparation of EF, the leaf samples were minced and macerated to the consistency of a fine paste by triturating repeatedly with 3% w/v distilled water. The processed material was stored in well-closed container at 2 °C as stock sample. This was administered as aqueous suspension for biological evaluations. Content uniformity of EF suspension divided doses was analyzed gravimetrically at two concentration levels. The mean deviation for 20 divided doses was found to be within ±4%.

Selection of Dose Levels for Biological Evaluation Considering dosage employed in ethnomedicinal practice, *i.e.*, *ca.* 5 g of macerated fresh leaves/adult woman/d,[3,4] the equivalent dosage for mature rats was calculated considering 10 times higher dose in comparison to humans.[24] Thus, the equivalent dose in rats was calculated to be 1 g/kg, considering average body weight of adult woman to be 50 kg. Both the test substances, AE and EF were initially administered at these dose levels. However, additional dose levels were selected in cases where the dose proved to be insignificant in any of the test groups in eliciting desired biological response or in circumstances where the dose level exhibited toxic symptoms during or following the period of application.

Evaluation of Post-Coital Pregnancy Interceptive Activity Female rats exhibiting normal estrus cycle were selected for the study. These female rats were caged overnight with coeval males of proven fertility in the ratio of 2 : 1 and the vaginal smears of female rats were checked on the following morning. The day of presence of spermatozoa in the vaginal smear was considered as day 1 of pregnancy. Mated rats were isolated, randomized into various treatment groups and treated orally with the test agent or distilled water (vehicle) during first 7 d of post-coitum. The animals were laparotomised under light ether anesthesia and sterile conditions on day 10 of pregnancy. Both horns of the uterus were observed for the number and status of implants and corpora lutea. The rats were allowed to recover and deliver after full term.[25]

Determining Variations in Estrus Cycle The estrus cycle of rats were monitored for 12 d in two groups, *viz.*, control and dose of test substance with best activity. Acyclic rats and rats with prolonged cycles were screened and eliminated. The process included examination of vaginal smear from each animal under microscope to observe different phases and duration of the estrus cycle.[26]

Estrogenic Activity and Biochemical Studies The test compounds and dose level exhibiting best activity profiles for post-coital pregnancy interception were subjected to an investigation for estrogenic activity and blood biochemical parameters.

Determination of Estrogenic Activity The immature female rats were randomly divided into groups of six animals each. The animals were treated once a day orally for 3 consecutive days with test agent, standard drug (0.02 mg/kg ethinylestradiol),[27] standard drug+test agent and only the vehicle. The animals were examined to observe premature opening of vagina and extent of vaginal cornifications 24 h after the last treatment and subsequently sacrificed. The uteri were removed, dissected free of adhering fat and blotted dry after expulsion of uterine fluid and the wet weights were recorded.[28] The uterine tissue (6 μm section) was subjected to histological examinations through haematoxylin–eosin staining.[27]

Blood Biochemical Studies The studies included investigation of serum gonadal and gonadotrophic hormones and lipid-carbohydrate profiles. 1.5 ml of blood was drawn from orbital plexus of adult female rats, before and after 8 d of o.d. treatment with the test agent and allowed to clot at room temperature for 30 min. The sample was then centrifuged at 1500 rpm for 15 min at 25 °C to obtain clear serum, which was aspirated out and frozen, until further analysis within 24 h. Serum gonadal hormones (progesterone, DHEA-S, estradiol and testosterone), gonadotrophic hormones (FSH and LH) and lipid-carbohydrate profiles (cholesterol, HDL, triglyceride and glucose) were estimated according to assay procedures specified by manufacturers of standard EIA, CLIA or enzymatic assay kits.

In principle, the process of gonadal hormone assay involves competition of hormone in sample with Horseradish peroxidase (HRP)-labeled hormone for binding to immobi-

lized antibodies on microwell plates. An enzyme substrate is added after washing the unbound substances. The amount of hormone in sample is inversely proportional to the enzyme activity. Adding a stop solution terminates the reaction and subsequently absorbance is measured in a plate reader.

Progesterone Assay[29,30] The sample (serum or standard) along with an enzyme conjugate (proteic buffer solution containing progesterone conjugated with HRP) were added in anti-progesterone IgG coated wells and incubated at 37 °C. After 1 h, the reaction solutions were aspirated, washed with water repeatedly and tetramethylbenzidine (TMB) substrate was added and incubated further for 15 min at room temperature, protected from light. The reaction was terminated by adding 1 N HCl (Stop solution) and absorbance was read at 450 nm within 30 min.

DHEA-S Assay[31] The enzyme conjugate containing DHEA-S conjugated with HRP and anti-IgG DHEA-S coated wells were used for estimation of DHEA-S by similar procedure followed for progesterone assay.

Estradiol Assay[29,32] The enzyme conjugate containing estradiol conjugated with HRP and anti-estradiol IgG coated wells were used for the reaction along with a competitive solution. The initial incubation was done for 2 h and the final incubation after adding TMB substrate was continued for 30 min instead. Rest of the procedure was similar to progesterone assay

Testosterone Assay[33] Similar to progesterone assay but enzyme conjugate contained testosterone conjugated with HRP and goat anti-rabbit IgG coated wells were implemented for the reaction along with a rabbit anti-testosterone reagent solution. The initial incubation was done for 1.5 h and the final incubation after adding TMB substrate was continued for 20 min instead.

In the assay of gonadotrophic hormones, the immobilization occurs during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously included biotinylated monoclonal anti-LH or FSH antibody. Upon blending monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction initiates between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration.

FSH Assay[34] The sample (serum or standard) along with FSH biotinylated/enzyme labeled antibodies is added to streptavidin coated microplate wells and incubated at room temperature. After 1 h the contents were aspirated, washed with wash-buffer (phosphate buffered saline with a surfactant) repeatedly and substrate solution (TMB+hydrogen peroxide) was added and incubated further for 15 min at room temperature. The reaction was terminated by adding 1 N HCl (Stop solution) and absorbance was read at 450 nm within 30 min.

LH Assay[35] Similar to FSH assay but LH-enzyme reagent was added instead of FSH biotinylated/enzyme labeled antibodies.

The lipid-carbohydrate parameters involved conventional enzyme specific colour reactions.

Chemical and Chromatographic Analysis The test substance AE was screened for presence of phenols, higher alcohols, steroids, essential oils, alkaloids, flavonoids, tannins, saponins, glycosides, sterols and terpenes by chemical analysis. Chromatographic sample preparation was done by extracting each sample (AE and EF) with either ethanol or ethyl acetate separately. The mobile phases used were *n*-butanol : methanol : water (3 : 1 : 1) and acetonitrile : water (1 : 1) for qualitative HPTLC or HPLC analyses respectively.

RESULTS

Evaluation of Post-Coital Pregnancy Interceptive Activity From the results (Table 1), it was observed that the test substance AE at 1 g/kg dose was ineffective in intercepting pregnancy and as such an elevated dose level was preferred to evaluate efficacy. A dose of 2 g/kg was also found to be ineffective in controlling pregnancy but the reduction in number of implantation sites with respect to control group was highly significant. The test substance, EF when administered at 1 g/kg for 6 d consecutively, caused behavioral changes and mortality. All the animals were found to expire within 8—11 d, and were autopsied to observe corpora lutea and implantation sites. No implantation sites were observed in any of the dead animals. The observable symptoms recorded in this group were i) decreased body weight and reduced food intake, ii) general weakness as revealed through slow and crippled movements and iii) mild respiratory depression following 3—4 d of drug administration. Due to this

Table 1. Contraceptive Property Assessment of Test Substances

Group	Test substance	Oral dose (mg/kg)	N	N_P	PI	n_{Cl}	n_I	n_L	n_{LD}
C	Vehicle	—	6	6	—	9.7±1.0	9.0±0.8	8.8±0.7	0.0±0.0
T_1	AE	2000	6	6	0.0	5.3±0.4	5.3±0.7	5.3±0.8	0.3±0.2
T_2	AE	1000	6	6	0.0	5.3±0.5	4.8±0.8	5.3±1.1	1.0±1.0
T_3	EF	1000	5	0	—	4.8±0.6	0.0±0.0	—	—
T_4	EF	500	9	3	66.7	6.4±0.9	2.6±1.3	2.1±1.1	0.3±0.2
T_5	EF	250	6	4	33.3	7.8±0.6	5.3±1.7	5.3±1.7	0.0±0.0

N, N_P, n_{Cl}, n_I, n_L, n_{LD} and PI represent the total number of animals in a group, total number of pregnant animals, number of corpora lutea, number of implantation sites, number of litters born on parturition, number of litters that died within 7 d of parturition and percentage pregnancy interception respectively. The results are presented as mean±standard error of mean (S.E.M.). One-way analyses of variances for n_{Cl}, $F=5.21$ [degrees of freedom (df)=5, 20], for n_I, $F=6.58$ (df=5, 20), for n_L, $F=5.96$ (df=4, 20) are significant at $p<0.01$ and for n_{LD}, $F=0.76$ (df=4, 20) is significant at $p>0.10$. The Least significant differences (at 5% level) are 3.290 (n_{Cl}), 5.098 (n_I), 3.593 (n_L) and 1.786 (n_{LD}); Ranked group means are [(C, T_5) (T_1, T_2, T_3, T_4) (T_4, T_5)] for n_{Cl}, [(C) (T_1, T_2, T_4, T_5) (T_3, T_4)] for n_I, [(C) (T_1, T_2, T_5) (T_4)] for n_L and [(C, T_1, T_2, T_4, T_5)] for n_{LD} and the two means not included in the same parentheses are statistically different at $p<0.05$.

shortfall, lower dose levels were selected for the experiment. A 500 mg/kg dose of EF resulted in 66.7% pregnancy interception and significant decrease in implantation sites without any behavioral changes and mortality. Some of the animals in this group exhibited implantation sites (Fig. 1) that did not deliver any litters on full-term. A dose level of 250 mg/kg of EF resulted in 33.3% reduction in pregnancy. Thus, EF at 500 mg/kg was adjudged to be the best dose for post-coital pregnancy interception among the test substances and dose level tested.

Variations in Estrus Cycle Significant ($p<0.05$, Student's *t*-test) shortening of the estrus cycle on treatment with EF at 500 mg/kg (4.3±0.1 d) has been observed in comparison to control group (5.5±0.5 d), $N=6$ (each group).

Estrogenic Activity and Biochemical Studies. Determination of Estrogenic Activity Results of study with EF at 500 mg/kg is delineated in Table 2. From the observations, it indicates that the treatment with EF at 500 mg/kg caused significant decrease in uterine wet weight as compared to control. None of the animals in this treated group exhibited estrus phase-like condition in vaginal smear examination. Histological observations (Fig. 2) reveal normal endometrial conditions in control group with minor cornifications, loose stroma and semi-prominent uterine glands. The tissue lumens of tested group were narrower with columnar epithelial cells in form of straight tubules, indicating terminal proliferative phase.

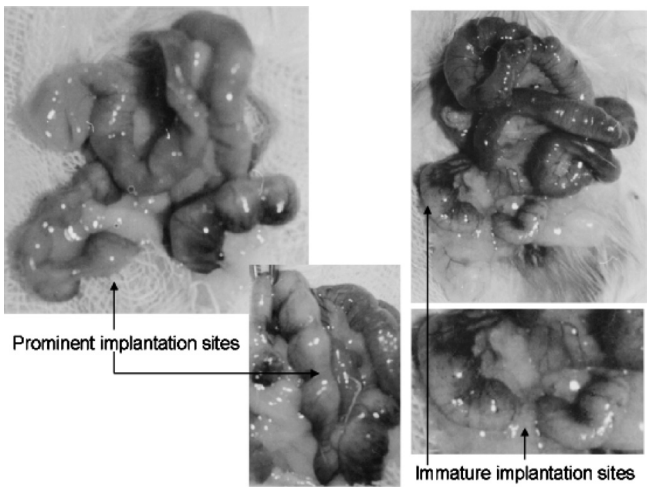

Fig. 1. Immature Implantation Sites at Uterus in EF Treated Group

Some animals in EF 500 mg/kg group exhibited such implantation sites that did not deliver any litter on full-term.

Blood Biochemical Studies Considering estrus cycle of EF (500 mg/kg) treated rats to be 4.3 d, blood was drawn post-treatment after 8 d following first drawn blood sample, so that phase-related changes in hormone levels get nullified while observing independent effect of drug substance. Table 3 depicts results of the biochemical assay.

There was diminution in serum progesterone levels (17.9%) and increments in serum estradiol (47.6%), DHEA-S (5.0%) and testosterone (32.5%) on treatment with the test agent (Table 3). With regards to gonadotrophic hormone assay, both FSH and LH were observed to be significantly elevated due to action of the test agent (Table 3). An appraisal of the associated changes in lipid-carbohydrate profiles (Table 3) following administration of EF revealed significant reduction in serum glucose (21.1%), cholesterol (15.0%), very large-density lipoprotein cholesterol (VLDL-C) (37.7%) and triglyceride (TG) contents (37.7%), while there was significant elevation of high-density lipoprotein cholesterols (HDL-C) (83.2%). The effect on low-density lipoprotein cholesterols (LDL-C) was not substantial (3.5%).

Chemical and Chromatographic Analysis Chemical examination of AE exhibited prominent presence of phenolic compounds, higher alcohols and saponins while moderate presence for tannins and traces of alkaloids, glycosides and terpenes. TLC (Table 4) of ethanol and ethyl acetate extracts of EF and AE demonstrated marked chemical differences.

Chemicals resolved with R_f values 0.47—0.48, 0.58—0.59 and 0.76—0.77 were found to be common in TLC chromatogram of AE and EF at 254 nm visualizations, while in 366 nm visualization chemicals resolved with R_f values 0.16—0.17 and 0.60—0.61 were similar. The ethanolic ex-

Table 2. Estrogenic Activity of EF (500 mg/kg) as Compared to Standard Drug, Ethinyl Estradiol (20 μg/kg)

Group	Test substance	Oral dose (mg/kg)	N	N_{OV}	N_C	U (mg/100 g b.w.)
C	Vehicle	—	6	0	0	163.7±22.0
T	EF	500	6	2	0	89.8±15.7
S	Ethinyl estradiol	0.02	6	6	6	222.1±10.7
TS	EF+ethinyl estradiol	500+0.02	6	4	3	145.1±17.1

U represents uterine weight, N_{OV} is the number of animals with open vagina and N_C represents number of animals with predominantly cornified cells like estrus stage. One-way analysis of variance for uterine weight, $F=9.30$ (degree of freedom=3, 15), is significant at $p<0.01$; The Least significant difference (at 5% level) is 52.801; Ranked group means are (C, TS) (T) (S) and the two means not included in the same parenthesis are statistically different at $p<0.05$.

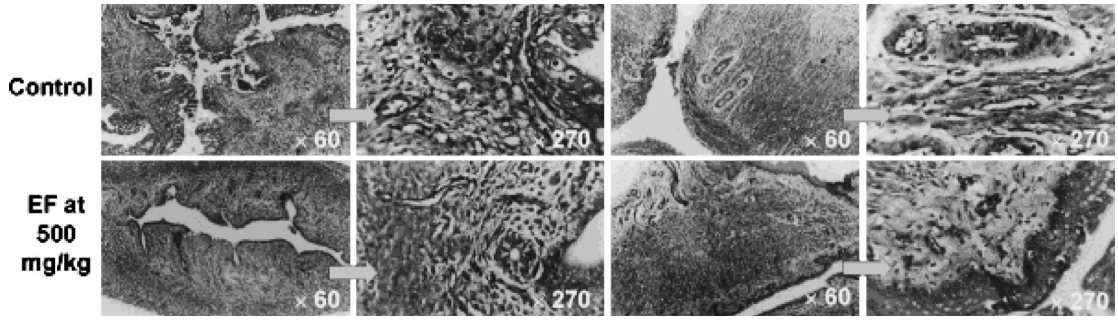

Fig. 2. Histology of Uterine Tissues

Minor cornifications, loose stroma and semi-prominent uterine glands observed in control group while narrow tissue lumens and columnar epithelial cells in form of straight tubules in tested group.

Table 3. Hormone, Glucose and Lipid Profiles of Blood on Treatment with EF (500 mg/kg)

Parameter	*N*	B.T.	A.T.	Unit	Percentage change[a)]
Progesterone	14	9.1±0.6	7.5±0.9	ng/ml	−17.9*
DHEA-S	14	7.5±1.4	7.9±1.0	ng/ml	5.0**
Estradiol	14	125.0±17.7	184.4±10.5	pg/ml	47.6*
Testosterone	6	3.2±0.1	4.3±0.6	ng/ml	32.5**
LH	14	0.7±0.1	2.8±0.3	mIU/ml	300.0*
FSH	14	0.6±0.1	1.3±0.2	mIU/ml	116.7*
Glucose	14	88.9±9.9	70.1±4.7	mg/dl	−21.1*
Cholesterol	14	28.9±5.5	24.6±4.6	mg/dl	−15.0*
HDL-C	14	2.8±1.0	5.1±1.0	mg/dl	83.2*
LDL-C	14	7.8±4.2	8.1±3.5	mg/dl	3.5**
VLDL-C	14	18.3±2.6	11.4±2.2	mg/dl	−37.7*
TG	14	91.7±13.0	57.1±11.0	mg/dl	−37.7*
Body weight	14	171.2±3.2	179.6±3.5	g	4.9*

B.T.: before treatment; A.T.: after treatment; *a*) Changes with respect to B.T. values and significant at $*\ p<0.05$ and $**\ p>0.05$ (pair wise Student's *t*-test).

tracts of both test materials were employed for subsequent HPLC analysis (Fig. 3), because the extracts of the same in ethanol exhibited comparatively better resolution profiles in TLC. The HPLC chromatograms showed a significant difference in chemical ingredients eluting at RT 8 min.

DISCUSSION

In the present investigation, leaves of tape-vine plant were evaluated for anti-implantation and related estrogenic properties. Studies on histological examination of isolated uterine tissues and blood-biochemical analysis were also taken up to explore possible mechanistic aspect for observed reproductive effects. Qualitative chemical assay of the test substances have revealed differences in comparative chemical parameters that might be responsible for the biological activity.

Among the test substances evaluated at different dose levels, AE at 1 and 2 g/kg and EF at 500 mg/kg in particular

Table 4. TLC Profile of EF and AE at 254 and 366 nm

Spots	254 nm								366 nm							
	U1		U2		U3		U4		U1		U2		U3		U4	
	R_f	A	R_f	A	R_f	A	R_f	A	R_f	A	R_f	A	R_f	A	R_f	A
1	—		—		—		—		—		0.03	151.3	—		0.03	1315.0
2	—		—		0.06	414.4	—		—		—		—		—	
3	—		—		—		—		—		—		—		0.09	106.4
4	—		—		0.10	1610.0	—		—		—		—		—	
5	—		—		—		—		—		—		—		0.13	427.7
6	0.15	244.1	—		—		—		—		—		—		—	
7	—		—		—		0.16	210.8	—		—		0.16	4024.3	—	
8	—		—		—		—		0.17	21.85	—		—		—	
9	—		—		—		—		—		—		0.18	1110.9	—	
10	—		—		—		—		—		—		0.21	4729.5	—	
11	—		—		0.22	1634.9	—		—		—		—		0.22	884.0
12	—		—		—		—		0.27	19.25	—		—		0.27	2469.0
13	—		—		0.29	2970.9	—		—		—		—		—	
14	—		—		—		—		—		—		0.30	2193.5	—	
15	—		—		0.34	3846.6	—		—		—		0.34	3347.0	—	
16	—		—		—		—		—		—		0.37	2481.2	—	
17	—		—		0.38	4097.2	—		—		—		—		—	
18	—		—		—		—		0.40	22.72	—		—		—	
19	—		—		—		—		—		—		0.43	3157.0	—	
20	—		0.44	540.2	—		—		—		—		—		—	
21	—		—		0.45	100076.3	—		—		—		—		—	
22	0.47	834.1	—		—		—		—		—		—		—	
23	—		0.48	333.0	0.48	5751.4	0.48	191.4	—		—		—		—	
24	—		—		—		—		—		—		0.49	11947.4	—	
25	—		—		0.51	4074.6	—		—		—		—		—	
26	—		—		—		0.53	1185.6	—		—		—		—	
27	—		—		—		—		—		—		—		0.55	2206.0
28	0.57	315.0	—		—		—		—		—		—		—	
29	0.58	366.9	—		0.58	17802.4	—		—		—		—		—	
30	—		0.59	371.3	—		0.59	3243.8	—		—		—		—	
31	—		—		—		—		—		—		0.60	16731.3	—	
32	—		—		—		—		0.61	36.18	—		—		—	
33	—		—		—		0.67	3215.4	—		—		—		—	
34	—		—		0.68	4300.9	—		—		—		—		0.68	1947.1
35	—		—		—		—				0.72	148.9	—		—	
36	—		—		0.75	2144.0	—		—		—		—		—	
37	0.76	1339.5	0.76	658.2	—		—		—		—		—		—	
38	—		—				0.77	910.1							—	
39	—		—		0.86	1336.1	—		—		—		—		—	
40					0.87	1100.6	0.87	3226.6	—		—		0.87	1332.5	0.87	1219.5

R_f : resolution factor; A: area under curve.

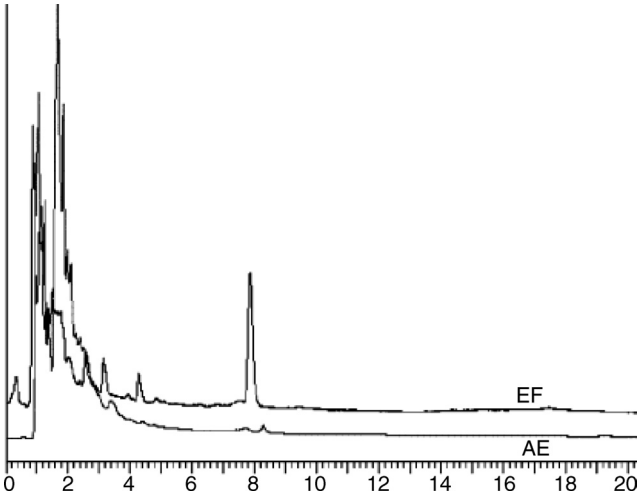

Fig. 3. HPLC Chromatograms of EF and AE at 254 nm
Observed difference in chemical ingredients eluting at RT 8 min.

were significantly potent in anti-implantation activity. There was complete absence of implantation sites in EF treated with 1 g/kg dose, but due to apparent toxicity of the dose level its pregnancy interceptive activity could not be ascertained. Toxicity observed in this dose level might be due to marked hypoglycemic effect of EF at 1 g/kg dose, as significant reduction in blood-glucose parameter (21.1%, $p<0.05$) was recorded on treatment with the same at 500 mg/kg. Even though 1 g/kg EF was the ethno-therapeutically equivalent dosage but the factor concerning co-administration of sugar or honey in consumption of EF, as practiced by ethnic societies[3] was not considered in the present experimental design. It is possible that this ethnic practice was to mask the strong hypoglycemic effect of this material.

There was significant reduction in number of litters born in comparison to control with AE at 2 g/kg and EF at 500 mg/kg. With regards to pregnancy interceptive capability of the test substances, none of the dose levels of AE produced desirable effect but EF at 500 and 250 mg/kg doses resulted in 66.7% and 33.3% pregnancy interception respectively. Significant reductions in number of corpora lutea in AE treated group might be due to decreased progesterone levels. Diminished progesterone levels are also responsible for restriction of implantation, as low progesterone levels can't retard the spontaneous rhythmic myometrial contractions of the uterus.[36] In contrast, EF at 500 mg/kg showed comparatively insignificant reduction in number of corpora lutea but significant reduction in implantation sites and number of litters born as well. This suggests that activity of EF may not be due to interference of ovulatory mechanism or abortive effects but possibly and principally anti-implantation in nature. There was insignificant number of deaths of newborn pups in all treatment groups.

EF at 500 mg/kg dose exhibiting 66.7% pregnancy interception demonstrated anti-estrogenic activity as revealed through significant decrease in uterine weight and absence of vaginal cornifications, while the same when co-administered with the standard estrogen, showed negligible change in uterine weight, likely due to opposite nature of the substances. It may be possible that chemicals present in EF antagonistically compete for the estradiol receptors (ER) in uterine tissues. Phenolic compounds have been explored through chemical examination in the present study that are also reported to be effective estradiol receptor competitors.[37] It is known that anti-estrogenic substances from natural sources decrease uterine wet weight.[38] Histological examination of uterine tissues also exhibited constricted cellular morphology in EF treated group, portraying anti-estrogenic conditions. Significant shortening of the estrus cycle on treatment with EF at 500 mg/kg dose has also been observed, suggesting mechanism of accelerated tubal transport.[39]

Significant decline in progesterone levels and marked increase in estradiol content following administration of EF 500 mg/kg dose, as revealed through blood-hormone level estimations, further substantiates the shortening of estrus cycle phenomenon. It is known that estrogens accelerate and progesterone retard the oviductal embryo transport.[40,41] Decline in progesterone levels may also cause conditions hostile to endometrial receptivity and maintenance of the blastocyst. Insignificant changes in serum DHEA-S or testosterone levels suggest non-interference of the test substance in biosynthetic process of estradiol. Rise in estradiol content of blood *per se* is possibly due to antagonistic interference of test substance with uterine estradiol receptors (ER_{α})[42] while causing agonistic effect on remote estradiol receptors (ER_{β}) known to be present in areas like the brain, bone and vascular epithelium, where estradiol have protective effects.[42] Groups of plant-derived compounds called phytoestrogens are known to bind with ER_{β}[43] and phenolic compounds are investigated to be one kind of potential phytoestrogen.[37] Thus, presence of phenolic compounds in the leaves of tape-vine plant, as revealed through chemical studies could possibly account for this effect. Significant surge of LH and FSH observed could be due to feedback effect of estradiol on hypothalamus to stimulate release of LH-RF and FSH-RF. Increased FSH and LH accelerates follicular and ovulatory phases to facilitate release of ovum. So, it is further confirmed that EF does not have any effect on inhibition of ovulation process. From Table 3, it can be ascertained that there is a significant decrease in blood glucose following treatment with EF at 500 mg/kg that possibly disrupts oxidative energy metabolism in uterus during implantation, providing environment unreceptive for implantation.[44] Significant decline in blood-cholesterol, VLDL-cholesterol, TG and increase in HDL cholesterol parameters proves that EF has favorable effect on lipid profile and seems to be free of cardiovascular risk factors, unlike marketed steroidal oral contraceptives.

TLC and chromatogram profiles reveal grossly dissimilar chemical pattern in both test substances, EF and AE. Chemical ingredients of alcoholic extract of EF eluting at RT 8 min in HPLC, markedly differed in chromatogram profile of AE. This component may additionally be isolated and tested for pregnancy interceptive activities. Moreover, the chromatograms developed may serve as qualitative chemical reference standards for these test substances.

CONCLUSION

The present study indicates that the ethnomedicinal formulation of tape-vine leaves has some post-coital pregnancy interceptive activity in female rats and the mechanism of action possibly involves anti-implantation activity by accelerated

tubal transport, and altered gonadal hormone levels. There is also likely disruption of endometrial ambience through decline in blood glucose parameter. The study did not find any correlation between probable anti-ovulatory mechanisms and observed reproductive effect, so the bioactivity is not due to interference with ovulation process. The study also indicated favorable effects of EF on blood-lipid parameters. Further work need to be taken up on similar investigation of an ethanolic extract of tape-vine leaves or fractions isolated from it, as the same exhibited good resolution of chemical compounds in TLC analysis.

Acknowledgements The authors wish to thank All India Council for Technical Education, New Delhi (India) for providing funds under Research Promotion Schemes and to Central Research Institute (Ayurveda), Kolkata (India) for partial material support. Assistances received from Narcissus Medical Centre, Serampore (India) in preparing the histological slides and Anchrom, Mumbai (India) for HPTLC analysis are also gratefully acknowledged.

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