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Antifertility activity of hydro alcoholic extract of *Moringa concanensis* Nimmo: An ethnomedicines used by tribals of Nilgiris region in Tamilnadu

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SUMMARY

In the present study, the hydro alcoholic extract of *Moringa concanensis* and their different fractions were evaluated for it's anti implantation, abortifacient, estrogenic and antiestrogenic activity. Hydro alcoholic extract of *Moringa concanensis* has showed potent antiimplantation and abortifacient activity at 200 mg/kg and 400 mg/kg respectively and marked estrogenic activity when administered individually and anti estrogenic activity was observed when administered along with ethinyl estradiol (1 μg/rat/day) as well as their different fractions of *Moringa concanensis* showed significant antiimplantation and abortifacient activity at 100 mg/kg. Moreover, all tested fractions showed significant anti estrogenic activity when administered simultaneously with ethinyl estradiol.

Key words: *Moringa concanensis*; Antifertility activity; Antiimplantation study; Abortifacient study; Estrogenic study; Antiestrogenic study

INTRODUCTION

The plant *Moringa concanensis* Nimmo (Moringaceas), locally known as Kattumurungai by tribal peoples of Nilgiris hill region in Tamilnadu is a tree, glabrous except the young parts and inflorescence. Leaves 2 pinnate, reaching 45 cm long. Flowers in lax divarieate thinly pubescent panicles reaching 45 cm long, segent white, oblong reflexed. Petals yellow, veined with red, oblong or oblong spathulate, the lower about 1.5 cm long. Capsules straight, acutely triquetrous, slightly constricted between the seeds. Seeds white or pale yellow, 3 angled, 3 winged wings very thin, hyaline (Kirthikar and Basu 1985). The plant *Moringa concanensis* Nimmo has been widely used as antifertility agent for decades by tribals of Nilgiris hill region. The tribals of Nilgiris, the hill region of the western ghats in Tamilnadu, were known to practice traditional medicine and our interaction with these tribals have given us the leads to several research projects with the possible presence of a therapeutic rationale in their claims in their use. The dried bark extracts of *Moringa concanensis* product were used by the tribals as antifertility agents. (Dhanasekaran *et al.*, 1993). Earlier reports showed that the presence of ascorbic acid (Dogra *et al.*, 1975), myristic acid, palmitic acid, oleic acid, stearic acid, arachidic acid and linoleic acid (Verma et *al.*, 1976) from the fruits of *Moringa concanensis* and seed respectively. In the present study has therefore, been planned to carry out investigations on traditional/folklore plants of *Moringa concanensis* Nimmo relating to antifertility activity to provide scientific validation in various

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experimental models.

MATERIALS AND METHODS

Plant material

The plant materials *Moringa concanensis* Nimmo stem bark were collected from the hills of Barliar, Ootacamund, Nilgiris district Tamil Nadu, India. The plants were authenticated at Botanical Survey of India, Coimbatore, India. The voucher specimens are preserved in our laboratory for further reference. After authentication, the plant materials were dried under shade. After optimum drying, the materials were coarsely powdered separately and stored in well-closed containers for further use.

Extraction and fractionation

The plant materials were shade dried and powdered separately. All plant materials were passed through sieve no.40 and used for extraction. A weighed quantity of each of the plant powder was extracted with alcohol: water (1:1) by cold maceration. The extract was evaporated to dryness under reduced pressure and controlled temperature (40 - 50°C). The percentage yield of the extract is 12.49%. The hydro alcoholic extracts was taken and suspended in water and successively extracted with petroleum ether, chloroform, ethyl acetate and ethanol. Petroleum ether, chloroform, ethyl acetate and ethanol fractions were dried over anhydrous sodium sulphate and concentrated to dryness under reduced pressure and controlled temperature (40 - 50°C) using rotary vacuum evaporator. The percentage yield of petroleum ether, chloroform, ethyl acetate and ethanol is 1.12%, 2.05%, 5.27% and 3.08% respectively.

Animals

Adult albino rats (Wistar strain) of female weighing 150 - 200 g and male albino rats 200 - 250 g were used. They were housed in standard microlon boxes with standard laboratory diet and water *ad libitum*. The experimental protocol was approved by the Institutional animal ethics committee Jsscp/ IAEC/Ph.Cology/02/05.

Anti-implantation activity

This study was performed following the method of Khanna and Chaudhury (1968). Proven fertile female rats of Wistar strain weighing 150 - 200 g were screened for 2 - 3 oestrus cycles consecutively by examining the vaginal smear. The rats showed normal cycles for two successive examinations were selected for the study. The rats were divided into 11 groups with six female rats in each group. The rats in proestrus and oestrus stage were caged with fertile male in the ratio 3:1. The following day vaginal smears were examined and the appearance of sperm clog in the smears was recorded as day 1 of pregnancy. The first group served as control and received vehicle only and second to eleventh groups received hydro alcoholic extracts of *Moringa concanensis* (200 and 400 mg/kg) and petroleum ether, chloroform, ethyl acetate and ethanol fractions of *Moringa concanensis* (50 and 100 mg/kg) were dissolved in 0.5% carboxy methyl cellulose from day 1 of pregnancy to day 7 respectively. On 10^{th} day animals in all groups were laparatomised under thiopental sodium (45 mg/kg; Neon Lab, Mumbai, India) anesthesia and examined for number of implants. Then the abdomen was sutured and rats were allowed for complete the term.

Abortificient activity

This method was performed as described by Khanna and Chaudhury (1968). Rats at day 1 of pregnancy were divided into 11 groups consisting of six animals in each group. The first group served as control and received vehicle only and second to eleventh groups received hydro alcoholic extracts of *Moringa concanensis* (200 and 400 mg/kg) and petroleum ether, chloroform, ethyl acetate and ethanol fractions of *Moringa concanensis* (50 and 100 mg/kg) were dissolved in 0.5% carboxy methyl cellulose respectively from day 8^{th} to day 14^{th}. During the experiment animals were observed for vaginal bleeding and on 21^{st} day animals were

laparatomised under thiopental sodium anesthesia and observed for dead implants.

Estrogenic activity

The study was carried out according to the method prescribed by Hiremath and Hanumantha Rao (1990). Colony bred Wistar strain female immature rats on 21 - 23 days old weighing 150 – 200 g, were bilaterally ovarectomised by dorsolateral approach under thiopental sodium (45 mg/kg) anesthesia with sterile conditions. They were divided into 12 groups consisting of six animals in each group. The first group served as control received vehicle only (0.5% carboxy methyl cellulose) and second group received ethinyl estradiol (Zydus Cadila, India) in olive oil, 1 μg/rat per day, subcutaneously. The third to twelfth groups received hydro alcoholic extracts of *Moringa concanensis* 200 and 400 mg/kg, petroleum ether, chloroform, ethyl acetate and ethanol fractions 50 and 100 mg/kg respectively. All the above treatments were given for seven days. On the 8th day, the rats were sacrificed by excess dose of thiopental sodium anesthesia. The uteri dissected out and surrounding tissues were removed. The uteri were blotted on filter papers and weighed quickly on a sensitive balance and fixed in neutral formalin for histopathology observations.

Anti oestrogenic activity

The study was carried out according to the method prescribed by Hiremath and Hanumantha Rao (1990). Colony bred Wistar strain female immature rats, 21 - 23 days old weighing between 35 and 45 g, were bilaterally ovarectomised by dorsolateral approach under thiopental sodium (45 mg/kg) anesthesia with sterile conditions. They were divided into 12 groups consisting of six animals in each group. The first group served as control received vehicle only and second group received ethinyl estradiol in olive oil, 1 μg/rat per day, subcutaneously. The third to twelfth groups received hydro alcoholic extracts of *Moringa concanensis* 200 and 400 mg/kg in addition ethinyl estradiol in olive oil, 1 μg/rat per day and petroleum ether, chloroform, ethyl acetate and ethanol fractions 50 and 100 mg/kg in addition ethinyl estradiol in olive oil, 1 μg/rat per day respectively. All the above treatments were given for seven days. On the 8th day, the rats were sacrificed by excess dose of thiopental sodium anesthesia. The uteri were dissected out and surrounding tissues were removed. The uteri were blotted on filter papers and weighed quickly on a sensitive balance.

Statistical analysis

The results were expressed as mean ± S.E. and the significance were evaluated by Student's *t*-test compared with control (Woodson 1987).

RESULTS

Antiimplantation activity

The antiimplantation activity is expressed as the percentage of animals showing absence of implantations in uteri when laparotomised on day 10 of pregnancy. The hydro alcoholic extract of *Moringa concanensis* exhibited 46% antiimplantation activity at 400 mg/kg. Whereas *Moringa concanensis* at 200 mg/kg did not show any significant antiimplatation activity. The results are depicted in Table 1.

The different fractions of *Moringa concanensis* also revealed significant post coital antifertility efficacy evidenced by antiimplantation activity. Chloroform, petroleum ether and ethanol fractions showed 55%, 52% and 51% of antiimplantation activity respectively when compared with control. Ethyl acetate fraction exhibited least percentage (21% and 13%) at tested dose levels. The antiimplantation activity of chloroform, petroleum ether and ethanol fraction may be attributed to the presence of alkaloidal, steroidal, principles present in the stem bark of *Moringa concanensis*.

Abortifacient activity

The abortifacient activity of the hydro alcoholic extract of *Moringa concanensis* was evidenced by

Table 1. Post-coital antifertility activity of hydro alcoholic extract of *Moringa concanensis* (HAEMC) and different fractions in female albino rats

Treatment	Dose (mg/kg)	Number of implants sites (mean ± S.D.)	Number of litters born (mean ± S.D.)	Antiimplantation activity (%)
Control (0.5% Carboxy Methyl Cellulose)		7.25 ± 2.40	6.30 ± 2.14	-
HAEMC	200	5.62 ± 3.08	4.26 ± 2.05	22
HAEMC	400	3.85 ± 1.26*	3.30 ± 1.12*	46
Petroleum ether	50	5.50 ± 2.04	4.96 ± 2.04	23
Petroleum ether	100	3.40 ± 1.75*	3.28 ± 2.55*	52
Chloroform	50	4.52 ± 2.30	4.18 ± 1.00	36
Chloroform	100	3.15 ± 1.06*	2.50 ± 1.16**	55
Ethyl acetate	50	6.20 ± 3.42	5.10 ± 1.84	13
Ethyl acetate	100	5.64 ± 2.86	4.36 ± 2.60	21
Ethanol	50	5.82 ± 2.00	4.42 ± 1.40	18
Ethanol	100	3.47 ± 1.34*	3.33 ± 1.32*	51

Values are mean ± S.E. (n = 6); *$P < 0.001$ compared with control, **$P < 0.01$ vs control.

number of implants vs. number of litters with 35% resorptions at 400 mg/kg dose level and the dose at 200 mg/kg did not show significant abortifacient activity. Similarly the different fractions of *Moringa concanensis* revealed that, chloroform, petroleum ether and ethanol fractions exhibited 56%, 42% and 39% resorptions, respectively. It once again implying that active constituent presents in the petroleum ether and chloroform fraction namely steroids may have anti fertility role for this plant. The results are presented in Table 2. Vaginal bleeding was also observed in the animals treated with above said test samples and it was due to resorptions of implantation sites after the 10^{th} day of the pregnancy.

Estrogenic activity and anti estrogenic activity

The effect of the hydro alcoholic extract of *Moringa concanensis* and various fractions of the plants were

Table 2. Abortifacient activity of hydro alcoholic extract of *Moringa concanensis* (HAEMC) and different fractions in female albino rats

Treatment	Dose (mg/kg)	Period of treatment	Number of implants sites (mean ± S.E.)	Number of litters born (mean ± S.E.)	Resorption (%)
Control (0.5% Carboxy Methyl Cellulose)		D_8 - D_{14}	6.36 ± 2.10	6.18 ± 1.25	2
HAEMC	200	D_8 - D_{14}	6.13 ± 3.44	5.64 ± 2.64	7
HAEMC	400	D_8 - D_{14}	5.94 ± 3.52	3.85 ± 1.46	35
Petroleum ether	50	D_8 - D_{14}	6.37 ± 3.06	5.22 ± 1.10	18
Petroleum ether	100	D_8 - D_{14}	6.44 ± 2.35	3.72 ± 1.75*	42
Chloroform	50	D_8 - D_{14}	6.83 ± 2.18	4.82 ± 0.50	29
Chloroform	100	D_8 - D_{14}	7.05 ± 3.55	3.06 ± 1.00**	56
Ethyl acetate	50	D_8 - D_{14}	6.48 ± 3.32	6.12 ± 1.40	5
Ethyl acetate	100	D_8 - D_{14}	6.18 ± 2.40	5.21 ± 1.29	15
Ethanol	50	D_8 - D_{14}	6.92 ± 3.38	4.92 ± 0.48	28
Ethanol	100	D_8 - D_{14}	6.47 ± 2.60	3.93 ± 1.74*	39

Values are mean ± S.E. (n = 6); *$P < 0.05$ compared with control, **$P < 0.01$ vs control.

Table 3. Estrogenic activity of hydro alcoholic extract of *Moringa concanensis* (HAEMC) and different fractions in female albino rats

Treatment	Dose (mg/kg)	Uterine weight (mg/100 g body weight) (mean ± S.E.)	Vaginal cornification
Solvent control (0.5% Carboxy Methyl Cellulose)		72.0 ± 5.34	-
Ethinyl estradiol	1 μg/rat/day	380.16 ± 15.64**	+++
HAEMC	200	85.15 ± 8.62**	-
HAEMC	400	96.34 ± 9.75**	+
Petroleum ether	50	68.36 ± 8.35*	-
Petroleum ether	100	75.14 ± 10.10	-
Chloroform	50	70.98 ± 7.60*	-
Chloroform	100	82.16 ± 11.28	+
Ethyl acetate	50	72.44 ± 6.60*	-
Ethyl acetate	100	85.50 ± 10.52	-
Ethanol	50	89.93 ± 12.54	-
Ethanol	100	98.18 ± 8.36	++

Values are mean ± S.E. (n = 6); *$P < 0.05$ compared with control, **$P < 0.01$ vs control. –: Nil; +: nucleated epithelial cells; ++: nucleated and cornified cells; +++: cornified cells.

Table 4. Anti-Estrogenic activity of hydro alcoholic extract of *Moringa concanensis* (HAEMC) and different fractions in female albino rats

Treatment	Dose (mg/kg)	Uterine weight (mg/100 g body weight) (mean ± S.E.)	Vaginal cornification
Solvent control (0.5% Carboxy Methyl Cellulose)		72.0 ± 5.34	-
Ethinyl estradiol	1 μg/rat/day	370.38 ± 10.45**	+++
Ethinyl estradiol + HAEMC	1 μg/rat/day + 200	358.12 ± 12.03**	+++
Ethinyl estradiol + HAEMC	1 μg/rat/day + 400	325.20 ± 14.18**	+++
Ethinyl estradiol + Petroleum ether	1 μg/rat/day + 50	336.62 ± 10.42**	+++
Ethinyl estradiol + Petroleum ether	1 μg/rat/day + 100	329.16 ± 12.50**	+++
Ethinyl estradiol + Chloroform	1 μg/rat/day + 50	315.42 ± 7.46**	+++
Ethinyl estradiol + Chloroform	1 μg/rat/day + 100	289.14 ± 9.34**	+++
Ethinyl estradiol + Ethyl acetate	1 μg/rat/day + 50	376.75 ± 8.62**	+++
Ethinyl estradiol + Ethyl acetate	1 μg/rat/day + 100	360.67 ± 10.88**	+++
Ethinyl estradiol + Ethanol	1 μg/rat/day + 50	350.36 ± 6.79**	+++
Ethinyl estradiol + Ethanol	1 μg/rat/day + 100	316.13 ± 10.16**	+++

Values are mean ± S.E. (n = 6); **$P < 0.01$ vs control. -: Nil; +: nucleated epithelial cells; ++: nucleated and cornified cells; +++: cornified cells.

studied for estrogenic and anti estrogenic activity in bilaterally ovarectomized immature rats. Oral administration of hydro alcoholic extracts 200 and 400 mg/kg of *Moringa concanensis* and ethinyl estradiol (1 μg/rat/day) exhibited significant ($P < 0.05$, $P < 0.01$) estrogenic activity (Tables 3 and 4). The uterotrophic potency as shown in the animals treated with ethinyl estradiol was more than in the animals treated with *Moringa concanensis* at tested dose levels. The uteri of these rats were inflated and fluid filled, resembling the proestrus/estrous uterus. They also induced vaginal opening and the

smear showed proestrous or estrous conditions, while all other control rats showed closed vagina. The number of cornified cells in the vaginal smears was considerably higher (+ to +++) than those of the control animals (0 to +), but notably less when compared to ethinyl estradiol treated rats (+++).

Simultaneous administration of ethiny estradiol (1 μg/rat/day) and hydro alcoholic extract of *Moringa concanensis* at 200 and 400 mg/kg caused significant ($P < 0.05$) increase in uterine weight. But extent of uterotrophic response was less than that produced by ethinyl estradiol alone (Tables 3 and 4). It also caused inflated uteri and vaginal cornification. However, the extent of all these changes was less than that of ethinyl estradiol alone treated rats. It shows that the hydro alcoholic extract of *Moringa concanensis* may have anti estrogenic properties.

The various fractions of hydro alcoholic extract of *Moringa concanensis* did not show any uterotropic activity and vaginal cornification when treated alone at tested dose levels in Tables 3 and 4. While simultaneous administration of ethinyl estradiol (1 μg/rat/day) and petroleum ether, chloroform, ethyl acetate and ethanol fractions at 50 and 100 mg/kg caused significantly ($P < 0.05$) increase in uterine weight. The number of cornified cells in vaginal smears also was higher than the control rats (+++). It may indicate that different fractions also may possess anti estrogenic activity.

DISCUSSION

In the present study hydro alcoholic extract of *Moringa concanensis* and its different fractions were tested for it's anti implantation, abortifacient, estrogenic and antiestrogenic activity. The study also conducted for possible toxicity of the plant for 28 days repeated dose study, at dose level of 400 mg/kg. Hydro alcoholic extracts of *Moringa concanensis* has showed antiimplantation and abortifacient activity at tested dose level and also showed marked estrogenic activity when administered individually and anti estrogenic activity was observed when administered along with ethinyl estradiol (1 μg/rat/day). Among fractionated extracts of *Moringa concanensis* petroleum ether, chloroform and ethanol fractions showed significant antiimplantation and abortifacient activity at 100 mg/kg body weight. Moreover, all tested fractions showed significant anti estrogenic activity when administered simultaneously with ethinyl estradiol.

It is well known that for implantation exact equilibrium of estrogen and progesterone is essential and any disturbance in the level of these hormones may cause infertility (Psychoyos, 1966). The hormonal values usually disturbs the hormonal milieu in the uterus and provokes an infertility effect. Therefore, the anti-implantation activity of extracts and fractions may due to estrogenic activity, causing the expulsion of ova from the tube, disrupting the luteotrophic activity of the blastocyst (Anderson, 1972). Simultaneous administration of ethinyl estradiol and hydroalcoholic extract of *Moringa concanensis* as well as different fractions showed significant increase in the uterine weight when compared with control ($P < 0.01$).

Our preliminary phytochemical studies showed the presence of flavonoids, alkaloids and sterols in *Moringa concanensis*. Several reports revealed that alkaloids, flavonoids and coumarins have antifertility activity (Sudhir *et al.*, 2001) and also flavonoids isolated from *Striga lutea* and *Striga orobanchioides* possessed strong estrogenic and antifertility properties (Hiremath and Hanumanth Rao, 1990).

Therefore, the antifertility property of the *Moringa concanensis* might be due to the presence of alkaloids, terpenoids and flavonoids. Oral administration of *Moringa concanensis* at 400 mg/kg for 28 days did not show any significant toxicity in rats. Therefore the phytoproduct from this *Moringa concanensis* is safe for therapeutic use.

A dose dependent antifertility activity of *Moringa concanensis* was observed in our present report. The loss of implantation and increased percentage of resorptions may be due to their blastocytotoxic activity. In immature female rats both plants

exhibited estrogenic activity and when given along with ethinyl estradiol they exhibited antiestrogenic activity. Estrogen is necessary for implantation and nidation. Hence, the antiimplantation activity of these plants may be due to an imbalance in endogenous estrogen levels. Our present report revealed that hydroalcoholic extracts and petroleum ether, chloroform and ethanol fractions of *Moringa concanensis* have significant antifertility activity. It also supports with our HPTLC findings that the compounds with Rf value of 0.95 having maximum peak area in petroleum ether and chloroform fractions of the plants. The antifertility property of the *Moringa concanensis* may be due to an anti estrogenic property and the said activity is may be due to the presence of steroids, alkaloids, terpenoids and flavonoids in both the plants.

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