Contraceptive Effect of Tukhme Sun (Seeds of *Crotalaria juncea*) in Experimental Animals

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Abstract

ukhme Sun (Seeds of *Crotalaria juncea*) is one of the many plant drugs described in Unani literature to be useful in family planning. The powder of *Tukhme Sun* was studied for anti ovulatory effect in albino rats and rabbits and for anti implantation effect in albino rats. In the test for anti ovulatory effect in rats, all the animals were treated with 700 mg/kg of the test drug for 10 days and the vaginal smear was examined daily for estrus/diestrus phase. Absence of diestrus phase was taken as the sign of contraception. In another test the rabbits were treated with the test drug (400 mg/kg) for three days and thereafter they were administered Cupric acetate to induce ovulation. After 48 hours the animals were laparotomized and the bleeding points in the uterus were observed. Test for anti implantation activity was carried out in pregnant rats after 10 days of treatment. The two horns of the uterus were observed for implantation sites after laparotomizing the animals. *Tukhme Sun* exhibited inhibition of ovulation in 80% and 77% of rats and rabbits, respectively. It also induced anti implantation effect in 40% of the animals. The study demonstrated that *Crotalaria juncea* possesses substantial anti ovulatory and moderate anti implantation activity.

Key Words: Contraception, Anti ovulatory, Anti implantation, Antifertility, *Crotalaria juncea*

Introduction

The current rate of population increase is 1.2% per year and therefore an addition of 80 million people every year, to the existing world population of 7 billion is inevitable. This growth in human population is taxing the natural resources and directly or indirectly affecting the quality of life world over. Control of fertility through contraception is thought to be the most important mean to arrest the growth rate. However, United Nation Fund for Population has estimated that some 200 million women worldwide, especially in the poorest countries, still have an unmet need for effective contraception. Meeting their needs would cost about US$3.9 billion a year, and could prevent 23 million unplanned births, 22 million induced abortions, 142,000 pregnancy-related deaths (including 53,000 from unsafe abortions) and 1.4 million infant deaths (www.unfpa.org/rh/planning.htm). In this backdrop the development of safe and cheap birth control methods assume special significance and therefore continues to be the high priority area of research in both modern as well as traditional medicines. Studies on contraceptives of plant origin drugs are considered

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important because the synthetic hormonal contraceptives in current use produce some side effects. Interestingly plants possessing diverse types of constituents such as terpenoids, alkaloids, glycosides, phenols, and other compounds have been reported to possess contraceptive and abortive properties. Therefore a number of plant species have been described to possess anti fertility and contraceptive effect. In Unani medicine various methods such as coitus interruptus and vaginal douches along with a number of oral contraceptives have been suggested as means of preventing conception (Adil, 1969; Himes, 1963). However, very few drugs have been investigated for the described effect. *Tukhme Sun* (Seeds of *Crotalaria juncea* family Leguminoceae) has been described to be an important anti fertility drug of Unani medicine possessing emmenagogue, anti ovulatory, anti implantation and abortifacient activity (Dymock, 1890; Ghani, 1921; Qureshi, 1957). Further, it has been reported to possess both anti spermatogenic effect in men (Vijaykumar et al., 2004) and anti fertility activity in female (Prakash, 1985). Although various extracts of *Cratolaria juncea* seeds (Fig 1 and 2) have been subjected to scientific study for anti fertilty activity with the report of anti implantation and anti oestrogenic activity in ethanolic extract (Prakash et al., 1993) but the powder of whole seeds which is the preferred dosage form in Unani medicine has not been investigated. Secondly the anti ovulatory effect of whole drug or its extract has not been studied, and the anti implantation effect due to oestrogenic effect is not very valuable from the clinical point of view (Prakash et al., 1993). In view of the above therefore the powder of *Tukhme Sun* was studied for anti ovulatory and anti implantation activity in female animal models.

Fig. 1. Pods and Flowers of *Crotalaria juncea*

Fig. 2. Seeds of *Crotalaria juncea*

Material and methods

Test drug

Tukhme Sun (Seeds of *Crotalaria juncea* Linn) was procured from Govt.

Agricultural Farm Kuwarsi, Aligarh. The seeds were further authenticated by Prof. S.H. Afaq at the Department of Ilmul Advia, A.M.U., Aligarh. They were dried in air and then ground in an electric grinder so as to prepare the powder of 100 number. A voucher specimen of the seed has been deposited in the museum of department of Ilmul Advia.

The dose of the test drug was calculated by multiplying the human therapeutic dose by conversion factor of 7 for albino rats and by 4 for rabbits (Friedrich et al., 1966) and it was found to be 700 mg/kg and 400 mg/kg, respectively. A fresh suspension of the powdered drug was prepared in normal saline before administration to the animals every day.

Study for antiovulatory activity in albino rats

Method of Kamboj (1982) was adopted to study the anti ovulatory effect of *Tukhme sun* in albino rats.

Regularly cyclic adult female rats weighing 100-150 g were divided in to 2 groups of 6 animals each in such a way that the total weight of animals in both the groups was approximately the same. The animals in group I served as plain control and were given the normal saline while the animals in Group II were treated with the powder of *Tukhme Sun* in the dose of 700 mg/kg orally once a day for 10 days. The vaginal smear of the animals was examined daily for estrus /diestrous phase. The animals persistently showing diestrus phase were recorded as positive and the percentage of such animals in each group was calculated.

Study for anti ovulatory activity in rabbits

The test drug was studied for anti ovulatory activity in rabbits by the method of Chaudhury et al (1970).

Adult female rabbits weighing 1.3 – 1. 5 kg were kept in isolation for 21 days to ensure that they were not pregnant and to prevent the induction of ovulation by mating. After isolation the animals were divided in to 3 groups of 3 animals each, such that the total weight of the animals in various groups was approximately the same.

The animals in Group I served as plain control and were administered normal saline. The animals in group II served as standard control and were treated with Norethisterone (German Remedies) in the dose of 0.25 mg /kg once a day for 3 days by oral route with an intragastric soft rubber catheter. The animals

in Group III were treated with the powder of *Tukhme Sun* in the dose of 400 mg/kg orally once a day for 3 days. Thirty minutes after the administration of the last dose a freshly prepared solution of Cupric acetate (0.4%) was administered to each animal intravenously through the marginal ear vein at the dose of 4 mg/Kg to induce ovulation. To check the ovulation, laprotomy of the animals was carried out 48 hours after the Cupric Acetate administration under light ether anaesthesia and the number of bleeding points on each ovary was noted as the indicator of ovulation. The percentage reduction of ovulation in the animals of standard and test groups was determined.

Study for anti implantation activity

The test drug was studied for anti implantation activity in albino rats by the method of Khanna and Chaudhury (1968).

Adult female albino rats of known fertility weighing 125-150 g were used for the study. The vaginal smear of the animals was studied for selecting the proestrus phase. The selected animals were allowed to mate with male rats of proven fertility. Next morning the vaginal smear was examined for the evidence of copulation as shown by the presence of thick clumps of spermatozoa. This day was designated as 1st day of pregnancy.

The pregnant animals were divided in to 2 groups of 5 animals each. The animals in Group I served as plain control and received the normal saline, while the animals in Group II were treated with the powder of *Tukhme Sun* orally at a dose of 700 mg/kg once a day for 11 days.

The rats were laparotomized on the 12th day of pregnancy under light ether anaesthesia and the two horns of uterus were examined for implantation sites. The percentage of the animals showing no implantation sites was determined and the significance level was determined by Sudent's 't' test.

Table 1: Effect of *Tukhme Sun* on ovulation in rats

Group	No. of Rats used	Rats persistently showing diestrus phase	Rats showing regular cycle	% inhibition of ovulation
Control	10	0	10	0%
Tukhme Sun (700 mg/kg)	10	08	02	80%

Table 2: Effect of *Tukhme Sun* on ovulation in rabbits

Group	No. of Rabbits used	Rabbits showing ovulation point	Rabbits showing no ovulation point	% inhibition of ovulation
Control	3	3	0	0%
Tukhme Sun (400 mg/kg)	3	1	2	77%
Norethisterone (0.25 mg/kg)	3	0	3	100%

Table 3: Effect of *Tukhme Sun* on implantation in rats

	No. of rats used	No. of rats showing implantation sites on 12 days	No. of rats showing no implantation sites 12th day	No. of rats showing implantation sites in individual rats	No. of rats died	Mean +SE of implantation sites	Percentages of rats having no implantation sites on 12th day
Control	5	5	0	5, 6, 4, 6, 6	0	5.4 + 0.357	0
Tukhme Sun (700 mg/kg)	5	3	2	2, 0, 1, 0, 2	0	1 + 0.40*	40

$P < 0.02$

Observation and Results

Anti ovulatory effect in rat

The daily examination of vaginal smear revealed complete absence of diestrus phase in the animals of control group, while 80% animals of test group showed persistently diestrus phase indicating inhibition of ovulation (Table 1).

Anti ovulatory effect in rabbits

Forty eight hours after intravenous administration of Cupric acetate all the animals were laparotomized (surgical incision was given in to the abdominal wall) to explore the ovaries and to examine the bleeding points on them. Presence of bleeding points indicates the ovulation. All the animals in control group showed bleeding points in the ovaries (100% ovulation), while those in standard group did not show such points (100% inhibition of ovulation). However in the test group 77% animals were found to exhibit inhibition of ovulation (Table 2).

Anti implantation activity in rats

All the animals in the control were found to possess implantation sites indicating that 100% animals conceived and retained the pregnancy whereas 40% animals of test group demonstrated absence of implantation sites on the two horns of the uterus (Table 3).

Discussion

Powder of *Tukhme Sun* produced about 80% and 77% anti ovulatory effect in rat and rabbit models, respectively. Further, in the test for anti implantation activity 40% of animals did not show any implantation site suggesting a moderate anti implantation effect possessed by the test drug. Anti ovulatory effect was found to be fairly better as compared to anti implantation activity. The findings assume significance because the anti ovulatory effect is considered more important than anti implantation activity in respect of contraception (Garg et al., 1971). The study for anti ovulatory effect was conducted in two different experimental models and both the studies demonstrated almost similar degree of effect despite the difference of animal species. Thus the complementary findings of the two tests confirmed that the test drug possesses significant anti ovulatory effect. It was also found to produce moderate degree of anti implantation activity as 40% animals showed absence of implantation sites on the two horns of the uterus. However further studies in this regard are required to decide the possibility of anti zygotic and abortifacient activity (Bhodanker et al., 1974). All three tests demonstrated that the test drug possesses striking anti ovulatory and moderate anti implantation activity therefore this plant drug can be categorized as one of the important sources of anti fertility or contraceptive agent. The findings are inconsonance with the earlier reports demonstrating that the ethanolic extract of *Crotalaria juncea* produced anti implantation activity (Anonymous, 1994; Prakash et al., 1993). In another study, chromatographic fraction of ethanolic extract of *Crotalaria juncea* have been shown to possess significant anti ovulatory activity. Oral administration of the fraction caused an increase in estrus and metaestrus phases and decreased the diestrus phase (Vijaykumar, 2007). Although this report is in conformity with our findings but the ability of chemicals and the isolated compounds to produce toxicity raise the doubts about their therapeutic utility. The plant drugs are subjected to research and development mainly to provide a safe and effective substitute to the available chemicals. Use of isolated component from the plant drugs is simply an upending and will not serve the purpose of providing safe substitution to toxic chemicals. *Tukhme Sun* and other such contraceptives from natural

sources if used as a whole would be especially useful in those cases where hormonal contraceptive agents are contraindicated. A number of active constituents have been isolated from plant drugs such as Lithospermic acid, m-Xylohydroquinone, Coronaridine, Rutin, and Rottlerin have been shown to actually possess the contraceptive effect but often with toxic effect (Farnsworth et al., 1975). There are many plant drugs on the other hand, without a distinct chemical that have the anti fertility effect probably because of the natural configuration of their simple constituents. *Tukhme Sun* has been reported to possess mainly proteins, fibers, oil, fatty acids etc (Anonymous, 1994; Jain & Iqbal, 1989) still it has shown contraceptive effect; it suggests that plant drugs may act in its entirety without the ascendance of any major active constituent. Presence of such a constituent in most of the cases is so meager that it cannot induce major toxicity.

In the present study the whole seed was administered to the animals in powder form which is most acceptable and preferred dosage form of the herbal drugs. The whole drugs with its entire constituents and their natural configuration are supposed to be compatible with the body homeostasis and therefore cause least toxicity. By demonstration of significant anti ovulatory and moderate anti implantation effect the present study substantiate scientifically the Unani usage of the test drug as an anti fertility agent and it also showed the possible mechanism (anti ovulatory) of anti fertility effect therefore indicating the great potential of *Tukhme Sun* as a contraceptive agent. But further studies are warranted in this regard at different dose levels so as to maximize its contraceptive potential.

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