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Anti implantation and Pregnancy Interruption Activity of Japakusuma (Hibiscus rosa Sinensis) & its combinations in Albino Rats

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ABSTRACT

Population explosion has created grave setback in the economic growth and all-round human development in developing countries. Current pandemic population explosion demands an immediate betterment of new potential contraceptives Easy availability and acceptability of contraceptive help the poorer countries to cut down the population growth and all other nations to avoid maternal morbidity and mortality arising from unplanned pregnancies. Hormonal steroids or various forms of barrier designs are predominantly used contraceptives methods. Studies of many years have highlighted the unmet demand for safe, inexpensive and acceptable contraceptives to avoid unwanted pregnancies. Oral contraceptives have been mentioned in Ayurvedic classics like 1) Pippali (Piper longum), + Vidanga (Emblica ribes) + Tankana (Sodii Biboras). 2) Talisapatra (Taxus baccata) + Gairika (Hematite) with cold water 3) Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel) Experimental studies on above mentioned 1, 2 & 3 formulations have been proved as temporary contraceptive medicine at BLDEA's AVS PGCRC Ayurveda Mahavidyalaya Bijapur. To evaluate the permanent or long term temporary contraceptive effect of Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel) in combination with Pippali (Piper longum) Vidanga (Emblica ribes) and Tankana (Sodii Biboras) an attempt was made in this study & compared with Individual drug effect. Study was carried out by Choudary and Khanna method on 18 female, 36 male (for mating) albino rats. Pippali (Piper longum) Vidanga (Emblica ribes) Tankana (Sodii Biboras) Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel) formulation, Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel) and Propylene glycol, formed the materials. Single dose was administered on proestrous stage of rat oestrous cycle & observed for anti implantation & pregnancy interruption activity. Test groups have shown significant anti implantation & pregnancy interruption activity. Test drugs have demonstrated significant anti implantation & pregnancy interruption of early pregnancy. Contraceptive activity of Drug combination was temporary.

Keywords: Anti implantation, Pregnancy interruption activity, Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel), Pippali (Piper longum), Vidanga (Emblica ribes), Tankana (Sodii Biboras)

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INTRODUCTION

The currently population explosion is one of the biggest problem in the world. Its inevitable consequences are employment, education, housing, health care, economy and environment. Currently, world population crosses the 7 billion and increasing continuously day by day. Therefore Fertility regulating becomes issue of global health concern. There is a great need to support at individuals in family-planning since increasing growth rate of world's population caused negative impact on sustainable, economic growth and poverty increased especially in developing countries [1,2]. The quest for the oral contraceptive agent that can control human fertility is as old as recorded history[3]. Numerous herbs have been reportedly used historically by women to reduce fertility[4]. Their application as medicine dates back to prehistoric period. Considerable number of drugs used in modern medicine has figured in ancient manuscripts such as the Rigveda, the Bible, and the Quran[5]. The Ayurveda is a Upaveda of Atharvaveda one among the four Vedas. Vedas have revealed that the herbs, shrubs and trees have got life much before the modern life science said it. The ayurveda has laid a scientific foundation for such thinking. Scientific studies in experimental animals have confirmed the effects of some of these herbs, herbo – minerals in the reproductive system[6]. Herbal & herbo – minerals contraceptives offer alternatives for women who have problems with or lack access to modern contraceptives options particularly women living in the rural areas in developing nations with very high population like India, China, Africa (Nigeria) and Bangladesh[7]. In Ayurvedic classical texts like Yogaratnakar, Bhavaprakash, Bhaishajya Ratnavali etc oral contraceptives like 1) Pippali (Piper longum) +Vidanga (Emblica ribes)+Tankana (Sodii Biboras). [8,9,10] 2) Talisapatra (Taxus baccata) +Gairika (Hematite) with cold water [8,9,10] 3) Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel) are mentioned. [8,9,10] Experimental studies on above mentioned 1, 2 & 3 formulations have been proved as temporary contraceptive medicine at BLDEA's AVS, PGCRC, Ayurveda Mahavidyalaya, Bijapur. [11, 12, 13, 14]. But till today, not much research has been conducted to find a non surgical permanent contraceptive or long acting temporary contraceptive by which pregnancy can be prevented. In order to evaluate the contraceptive effect & to provide safe & economic contraceptive formulation, an attempt was made with this research project.

MATERIAL AND METHOD

Materials

Drugs: Pippali (Piper longum), Vidanga (Emblica ribes), Tankana (Sodii Biboras), Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel) Animals : Wister strain male & female albino rats

18 female, 36 male albino rats were taken from the animal house, BLDEA's AVS PGCRC Ayurveda mahavidyalaya Bijapur. Institutional ethical clearance no was AVS/PGCRC/IAEC/18/2007. All the experimental animals were maintained under standard laboratory conditions, fed with balanced food & water as per the CFTRI formula prepared at Pranav food industries Sangali, Maharashtra. 12 hour light & darkness maintained in animal house with temperature of 18^0-25^0C. Different groups of animals placed separately in propylene rat cage.

Method of preparation of Drug formulation: [8, 9, 10]

Raw Pippali (Piper longum) & Vidanga (Emblica ribes) collected separately and physical impurities were removed. Then subjected to pounding in pounding machine. Resultant powder sieved and fine powder collected in clean container.

Raw Tankana (Sodii Biboras) collected and purified according to Rasatarangini 13th chapter 75,76 sloka [15]

Japakusuma (Hibiscus rosa Sinensis) pushpa was taken in clean & dry Khalwa yantra, pounded well & fine powder was prepared by vastra galana method. Thus prepared fine powder was mixed with kanji (sour gruel)} in a motor & pestle and subjected to bhavana. (Trituration).

Powder of all 4 dravyas were mixed, uniform mixture was prepared & stored in clean container. Then used for phytochemical analysis & experimental study

Method of preparation of Medicine for administration:

Group I : Fine powder of Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel), Pippali (Piper longum), Vidanga (Emblica ribes) & Tankana (Sodii Biboras) were taken & mixed well into 2 ml of Propelyne glycol, shacked vigorously in test tube & then the uniform suspension was fed to albino rats orally by a syringe.

Group II: Fine powder of Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel), was mixed well into 2 ml of Propelyne glycol & shacked vigorously in test tube & was fed to albino rats orally by syringe.

Group III: Fine powder of Pippali (Piper longum), Vidanga (Emblica ribes) & Tankana (Sodii Biboras) were taken & mixed well into 2 ml of Propelyne glycol, shacked vigorously in test tube & then the uniform suspension was fed to albino rats orally by a syringe

Method of selection of Animals

Inclusion criteria:

Healthy fertile female albino rats of child bearing age & with normal oestrous cycle. Body weight between 150 to 200 grams. Fertile male rats were taken for mating.

Exclusion criteria:

Unhealthy albino rats, female albino rats of body weight less than 150 grams and more than 200grams. Sterile male & female rats.

Anti implantation activity

Anti-implantation activity was conducted by Choudary & Khanna Method It involves 6 stages [16-20]

a. Taking vaginal smear.

b. Examination of smear to know the phase of oestrous cycle.

c. Allowing animals for mating 1 : 2 (female : male) ratio.

d. Observation for sperm clumps to confirm mating.

e. Drug administration

f. On 10^{th} day of drug administration rats were subjected to laparotomy to observe for implantation

Sample size: n= 6 in each group

Drug schedule:

Group I: 180 mg / 200 gm body weight of albino rats test drug Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel) & 180 mg / 200 gm body weight of albino rats test drug Pippali (Piper longum) (60mg), Vidanga (Emblica ribes) (60mg) & Tankana (Sodii Biboras) (60mg) with 2 ml of Propelyne glycol

Group II: 180 mg / 200 gm body weight of albino rats test drug Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel) with 2 ml of Propelyne glycol

Group III: 180 mg / 200 gm body weight of albino rats test drug Pippali (Piper longum) (60mg), Vidanga (Emblica ribes) (60mg) & Tankana (Sodii Biboras) (60mg) with 2 ml of Propelyne glycol

Outcome measures

Primary outcome

To compare the anti implantation activity of Japakusum, Pippali (Piper longum), Vidanga (Emblica ribes) & Tankana (Sodii Biboras) to that of Japakusum and Pippali (Piper longum), Vidanga (Emblica ribes) & Tankana (Sodii Biboras)

Secondary outcome:

1. Mean birth weight of litters
2. Survival of litters

RESULTS AND DISCUSSION

Table 1: Phytochemical constituents of aqueous extract of Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel) & its combinations.

Sl. No.	Organic Constituents	PVTJ	J	PVT
1	Alkaloids	-ve	- ve	+ve
2	Carbohydrates	-ve	-ve	- ve
3	Tannins	+ve	+ve	+ve
4	Steroids	+ve	+ve	+ve
5	Triterpinoids	-ve	-ve	-ve
6	Saponins	+ve	+ve	+ve
7	Flavonoids	+ve	+ve	+ve
8	Caratoinoids	-ve	-ve	-ve

Note : **PVTJ**: Pippali (Piper longum), Vidanga (Emblica ribes) & Tankana (Sodii Biboras) & Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel)

J :Japakusuma

PVT : Pippali, Vidanga & Tankana

Table 2: Anti implantation activity of PVTJ (Pippali (Piper longum), Vidanga (Emblica ribes) Tankana (Sodii Biboras) Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel)), J (Japakusuma) & PVT (Pippali, Vidanga, Tankana) (primary outcome measure): (n=06)

Group	Drugs	No. of Rats	Mean no. of implantations	% inhibition of implants
I	PVTJ	6	0	100%
II	J	6	0	100%
III	PVT	6	0	100%

Note: 1) PVTJ: Pippali (Piper longum), Vidanga (Emblica ribes) & Tankana (Sodii Biboras) & Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel)

2) J: Japakusuma

3) PVT : Pippali, Vidanga & Tankana

All the animals in Test groups have demonstrated 0% mean number of implants & 100% inhibition of implants (anti-implantation).

Table 3: Secondary outcome measures of PVTJ (Pippali (Piper longum), Vidanga (Emblica ribes) Tankana (Sodii Biboras) Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel)), J (Japakusuma) & PVT (Pippali, Vidanga, Tankana). (n=06)

Group	Drugs	% of rats delivered on full term	Mean no births	Mean weight of litters	Died within 2 days
I	PVTJ	0%	0	0	0
II	J	0%	0	0	0
III	PVT	0%	0	0	0

Note: 1) PVTJ: Pippali (Piper longum), Vidanga (Emblica ribes) & Tankana (Sodii Biboras) & Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel)

2) J :Japakusuma

3) PVT : Pippali, Vidanga & Tankana

In all the groups & 0% of rat delivered on full term, mean number of births were 0 (zero), 0 (zero) mean weight of litters & 0 (zero) litters died within two days.

In this anti implantation & Pregnancy interruption study, results of test sample were compared. Study was conducted in six stages. To assess contraceptive activity of test sample by the observation of anti implantation & pregnancy interruption activity in mature female albino rats. In 1st stage, anti implantation activity was conducted to assess contraceptive activity by following Choudary & Khanna method. Laparotomy was conducted on 10th day after drug administration. Results of three groups were compared. In Group I, II & III implantations were not found, indicating all the drugs have demonstrated anti implantation & Pregnancy interruption activity. The anti implantation activity & pregnancy interruption activity might be postulated in the following ways based on the experimental & phytochemical studies. Estrogen and progesterone hormones are essential for maintenance of regular menstruation cycle, production of ovum, maintenance of pregnancy in all stages. Hence anti implantation activity seen in this study may be due to anti oestrogenic and anti progestrogenic effect of test drug. Phytochemical analysis of 1) Pippali (Piper longum), Vidanga (Emblica ribes) & Tankana (Sodii Biboras) & Japakusuma (Hibiscus rosa Sinensis) Kanji bhavita (Triturated with sour gruel) 2) Japakusuma 3) Pippali, Vidanga & Tankana have shown the presence of saponins, steroids, flavanoids & tannins. Especially steroids & saponins are used as raw material for preparation of medically useful steroids & sex hormones like progesterone, oestradiol, & testosterone. Thus steroids, saponins might have contributed in the contraceptive activity of the drugs. Significant anti implantation & pregnancy interruption activity was noted in all the Groups indicating the contraceptive

activity of the test drugs. The presence of Steroids, Saponins, flavanoids & Tannins might have contributed in contraceptive activity.

Scope for further research

This is an animal based experimental study. But the promising results of this study necessitate a well designed randomized clinical research before the test drug is recommended for clinical practice.

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