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Comparative Study of Poly-Herbal Formulations with Allopathic Drug for Abortifacient Activity on Wistar Rat

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ABSTRACT:

The population growth of past several years in India was due to difference in the number of birth rate and number of death rate. To control the birth rate, fertility control is required. There are many methods for fertility regulation available but the oral contraception method is most popular method for contraception. The traditional medicines are being popular in the current time because of having fewer side effects as compare to synthetic allopathic drugs. There are many herbal drugs having fertility regulating activity but they are not as effective as compare to Synthetic allopathic drugs. As per market survey, doctors opinion that ayurvedic abortion drugs are not effective in the process of abortion. While in current market ayurvedic abortion drugs are more used than the allopathic abortion drugs by patients. Hence scientifically designed research on ayurvedic abortifacients for its effectiveness in the abortion process to compare with standard allopathic drug like Mifepristone is highly required to save the public health. In this study it is confirmed that there is a no abortifacient activity seen in Polyherbal formulations in comparison of standard Mifepristone drug sold in today's market.

KEY WORDS: abortion, abortifacient, Mifepristone, Poly-herbal formulation, Contraceptive

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INTRODUCTION:

India is among the highest populated countries in the world. The latest population of India is 1.21 billion which showed the population growth rate 17.64 percent as compared to 2001. The number has increased by 181 million. Our population density is 382 people per square kilometer^[1]. By these data we concluded that there has been a significant increase in population of our country. The population of our country is rapidly increasing and unfortunately our natural resources like water, petroleum, minerals are in limited quantity. Other problems like waste plastics and other non-bio degradable materials since they once made then after it is difficult to degrade them and it is polluting the air, land and water. The increase in the number of vehicles and industries produced very critical damage to nature. The growth of population can cause critical damage to economy and social problems in our country.

Advances in public health and medicine have lead to decrease in the number of deaths and increase in the chances of life expectancy which also resulted in increasing population. Thus, for control the growth of populations there is only one option is to control the birth rate. And to control birth rate, fertility control is required, which can be controlled by contraceptives.

There are choice of many methods of contraception which will prevent pregnancy, including spermicidal alone, natural (rhythm) method, diaphragm or cap alone, condom alone, diaphragm with spermicidal, condom with spermicidal, intrauterine

device (IUD), contraceptive pill, sterilization (either a man or woman), avoiding intercourse at specific time. Among all of them, mostly used methods for contraception are synthetic oral contraceptive pills because they don't required technically skilled personnel^[2]. Several clinical researchers have suggested that the abortion pill is a good solution for populations developing countries where skilled medical and surgical personnel are in short supply and also in the remote and rural areas where less frequency of hospitals and clinics^[3].

On the other side of advantages, many women experience emotional or physical adverse effects when taking oral contraceptives^[4]. Despite the serious impact on women's health, there is absence of valid techniques to screen women for their risk of oral contraceptive side effects^[5]. It increases the oxidative stress and produces many serious side effects such as breast cancer and cervical cancer^[6]. Oral contraception can also cause hypertension especially after the 5th year of use chances to be 2.5-3 times higher than for the first year. It is concluded that use of oral contraceptives can induce risk of thrombosis and of thrombophlebitis 4-times to 6-times increase among woman over 35^[7]. Contraceptive use before a surgical procedure can increase the risk of postsurgical thromboembolism. A chance of cerebral thrombosis, myocardial infarction is also higher among Oral contraceptive users^[8].

Our country requires regular affordable essential Drugs. For these populations, modern medicine is not to be a realistic treatment option. In other way traditional medicine is widely available and affordable, even in remote and rural areas. Traditional medicine is generally accessible to most people. It is important for primary health care centers and its availability is widespread in developing countries^[9].

The plant products are now becoming more popular than the synthetic allopathic drugs due to their low toxicity and long standing experience of use in the traditional literatures like the Ayurveda. Due to side effects of synthetic steroidal contraceptives, interest has been focused on the indigenous plants for possible contraceptive effect. Although the contraceptives containing estrogen and progesterone are effective and popular, due to the risk associated to these drugs have increased the need for suitable product from indigenous medicinal plants that can be used as alternative of pills. One of the greatest arguments against traditional medicine is the lack of scientific proof of its effectiveness. There is insufficient scientific investigation on most of the claims made by the traditional medicine practitioners^[10]. There is another problem of Safety. People think that traditional herbs are safe and harmless since they are natural and are not invented in the

laboratory. But use of these herbs may possibly expose the patient to unknown dangers [11, 12, 13]. For example aristolochic acid and other components within herbs can cause adverse renal effects and renal toxicity [14]. Some herbal remedies used to treat liver disease may be hepatotoxic themselves [15].

There is also possible serious reaction between herbal therapy and biomedical medications. A survey says that most patients do not inform their physician that they are taking herbal treatment. This may result in serious and fatal reaction. The most common published and worst herb drug reaction is of St John's wort (*Hypericum perforatum*) and drugs that are metabolized by cytochrome P450, CYP 3A4 isoenzymes used without informing their physicians patients use St John's wort to treat depression. This may have varies adverse effects. The woman taking oral contraceptives with St John's wort may cause breakthrough menstrual bleeding, unexpected pregnancy. Other problem with traditional medicine is the lack of hygiene and precise dosage^[16].

An extensive literature review suggested that there was already several scientific research papers have been published related to fertility control from herbs^[17], but there was not significant effectiveness of any single plant to be used alone in place of birth control pill^[18]. The combination of these herbal drugs may have significant effectiveness as compare to single plant.

There were many Poly-herbal formulations which were available in the market but having no scientific proof of their activity. So our research work was aimed to search for the marketed but not scientifically proved poly-herbal formulation and to find out its effect on fertility as compared to standard oral contraceptives.

In this research work, we were going to evaluate and compare the efficacy of the traditional herbal drugs with standard synthetic drug. We funded many poly-herbal drug products but the most widely used and popular products were selected to evaluate the antifertility effect.

The two marketed preparations (Product-A and Product-B) were selected. Prduct-A contains *Daucus Carota*, *Clerodendron Serratum*, *Ferula Narthex*, *Aloes Indica*, and *Trikata*. Product-B contains *Aloe Indica*, *Crocus Sativas*, Carrot seed, *Jund Badster*, *Gossy Piam*, *Raphanus Sativas*, Ferrous, *Bathua* juice. The review of literature suggested that the herbal constituents of the given formulation were containing the antifertility drugs as well as some beneficial herbal constituents which are having preventive action against the side effects like Anemia, Cancer,

Diabetes, Cardiac disorders which can probably occurred due to the antifertility drug treatment. The Mifepristone drug was obtained as the active pharmaceutical ingredient from Biocon Pharmaceuticals, Bangalore (Karnataka) and it was taken as a standard drug which is most commonly prescribed drug for prevention of birth by many Physicians.

The main objective of our study was to compare the abortifacient activity of the polyherbal formulations Product-A and Product-B with standard Mifepristone drug in a scientific manner using suitable experimental model.

MATERIALS AND METHODS

Drugs and Chemicals

The objective of the study was to check Abortifacient potential of the poly herbal formulations and compare it with standard Mifepristone drug. Mifepristone in purified powder form was obtained as a free sample from Biocon Ltd., 20th K.M. Housur Road, Bangalore, Product-A and Product-B Strips were obtained from the local market of Rajkot (Gujarat).

The detailed information of content of the polyherbal formulations:

A. Product-A (Capsule):

Each capsule contains:

Daucus Carota.....	300mg
Clerodendron Serratum.....	50mg
Ferulla Narthex.....	50mg
Aloes Indica.....	50mg
Trikatu.....	50mg

Dosage: As directed by the physician

Caution: Not to be given during pregnancy

B. Product-B (Tablet):

Each uncoated tablet contains:

Aloe.....	50mg
Crocus Sativas.....	2.74mg
Carrot seed.....	60mg
Jund Badster.....	2.74mg
Gossy Piam.....	60mg
Raphanus Sativas.....	60mg
Ferrous.....	60mg
Bathua ka Ras.....	q.s.

Dosage: One tablet twice a day.

Animals Study Protocol

Wistar albino rats of either sex were used for this study. The animals were procured and housed in the animal house of School of Pharmacy, RK University. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Six rats were taken for each group. The rats were acclimated to the laboratory environment for 7 days prior to the study. Animal house was well maintained under standard hygienic condition, relative humidity of 44%-55%, 12 hours day and night cycle, Air renewal was approximately 13 times per hour. Animals were fed in rat chow and water ad libitum.^[19,20,21]

All the experimental procedures and protocols used in study was reviewed and approved by the Institutional Animal Ethical Committee (IAEC) and care of laboratory animals were taken as per the guidelines of Committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India. (IAEC Protocol number: **RKCP/COL/RP/13/37**)

Dose Preparation for Animals

Assuming the dose for abortifacient activity requirement of rats (250-300 mg. average weight) is fixed on women dose (60 kg. average body weight) was calculated. The appropriate doses were prepared by triturating and suspending the drug powder in water using 1% Sodium Carboxymethyl Cellulose as suspending agent. The triturated drug powder was suspended in 1% Sodium Carboxymethyl Cellulose. Female rats were weighed and allocated to the four groups to give approximately equal group mean body weight. The oral dosing was done by oral feeding needle. Group I received vehicle only (Distilled Water) and served as the control. Groups II, III and IV received Mifepristone Oral suspension (3.3mg/kg), Product-A Oral Suspension (8.3 mg/kg) and Product-B Oral Suspension (5.0 mg/kg) respectively. The formulation was prepared in such a way that it can be given to the animals 1ml twice a day. The dose was given by oral feeding needle.

Experimental Procedure for taking Vaginal Smears^[22]

A small amount (approximately 0.2 ml) of saline or distilled water was drawn up into the Plastic Dropper tip. The rat was hold around the thorax, ventral surface uppermost, with one hand whilst the hand holding the Dropper was used to restrain the tail, to provide additional support and help prevent the animal struggling. The tip of the pipette was pushed gently into the entrance of the vagina to a depth of 2-5 mm and the fluid was flushed into the vagina and back up into the pipette two or three times by gently squeezing and releasing the bulb of the pipette. The flushing was repeated until the fluid was

seen to be cloudy. The smears were fixed by placing the slide in absolute alcohol for 5 sec, allowing it to dry, and staining it with a 5% aqueous- methylene blue solution for 10 min. The excess stain was washed off with tap water, and the slide was dried and observed using the low power (10X) and High power (45X) of a microscope. Staining was recommended because it is easy and quick to do, and greatly facilitates the scoring of the smears.

Detection of Mating ^[23]

Mating was detected by the presence of a milky white to yellow, waxy vaginal plug due to secretions from the male's coagulation glands. These plugs normally persisted 12 - 24 hours and after discharge found in the cage or litter pan. If timed pregnancies are required by the investigator, the rats should be checked daily for the presence of a vaginal plug. Mating can also be confirmed by the presence of sperm in the vaginal smear.

Basic Ultrasound Imaging ^[24-33]

Principle

Ultrasounds are the sound waves that are not detectable by the human ear with frequencies greater than 20,000 cycles/sec (Hz). Diagnostic ultrasound commonly uses frequencies between 2 and 15 MHz (106 cycles/sec). Intravascular transducers commonly use frequencies up to 30 MHz, and ultrasound biomicroscopy systems with transducers using frequencies up to 100 MHz have been reported^[34]. At these frequencies, sound waves are transmitted through soft tissue relative to the acoustic impedance of each tissue. The acoustic impedance of a particular tissue is the product of the transmission velocity of sound and the tissue density. The transmission velocity in most soft tissues and blood is nearly uniform at 1540 m/sec^[35]. Therefore, the acoustic impedance of most soft tissue is primarily a function of the tissue density. When two tissues with different densities are located next to each other, an acoustic impedance mismatch is created and sound waves are reflected by the mismatch.

The greater the acoustic mismatch or difference in tissue densities, the more sound waves are reflected and returned to the transducer. Areas with relatively large tissue density differences, and hence more reflected sound waves, are generally seen as brighter areas on the final image. As a result of these properties, ultrasound imaging is best suited for soft tissue imaging and is often limited by bone- and gas-filled structures. Sound is transmitted rapidly through bone tissue. Conversely, sound is poorly transmitted through air and air filled structures. The large acoustic impedance mismatch that

occurs at bone and gas interfaces with soft tissue causes the majority of sound waves to be reflected. This large reflection decreases sound wave penetration into deeper tissues and can cause imaging artifacts. Despite this limitation, specialized techniques have been developed to perform transcranial Doppler imaging of the cerebral vasculature in humans and dogs [36].

Resolution or the ability to distinguish two closely situated structures or events accurately is an important concern for all imaging methodologies. However, resolution becomes even more important when imaging small targets such as rat and mouse organs. For example, the left ventricular (LV1) chamber diameter of a mouse heart is 2 to 3 mm and the LV posterior wall is approximately 0.6 mm thick at end diastole [37]. In rodent ultrasound imaging, both spatial and temporal resolution must be considered. Spatial resolution is further divided into axial and lateral resolution.

Axial resolution is the ability to distinguish two separate but closely positioned structures situated parallel to the propagation axis of the ultrasound beam. Axial resolution is dependent on sound wave pulse length and frequency (wavelength). Two structures will be seen as separate structures only if the pulse length is shorter than the distance between the structures. If the pulse length is longer than the distance between the structures, only a single reflection will be detected and the image will show only one structure. Higher frequency sound waves have shorter pulse lengths and generally greater axial resolution.

Lateral resolution refers to the ability to distinguish two adjacent but separate structures oriented perpendicularly to the axis of the sound wave beam. Lateral resolution is dependent on beam width and sound wave frequency^[38]. Narrower beam width provides greater lateral resolution. Beam width can be minimized by focusing the sound waves as they are produced by the transducer. In most ultrasound systems, beam focusing is performed by curving the elements of the transducer or by electronically controlling the elements of the transducer. Lateral resolution is also influenced by the frequency of the sound wave, with higher frequencies generally improving resolution.

Because axial and lateral resolution improves with increasing frequency, higher frequency transducers are generally preferred for rat and mouse imaging. Rat hearts have been imaged using a variety of ultrasound systems with transducers operating at frequencies from 5 to 12 MHz. Transducers that image at frequencies above 9 MHz are commonly used to image mouse. Utilizing very high frequencies and narrow beam widths, UBM systems with 40- to 60-MHz transducers can have spatial resolution approaching 50. One limitation of

using higher frequencies is that as the frequency increases, sound wave penetration decreases. For example, a 5-MHz transducer will generally image to a depth of 12 to 15 cm, but a 10-MHz transducer may image to a depth of only 3 to 4 cm. The higher frequency UBM systems may penetrate only 1 to 2 cm[39]. hence, increasing axial and lateral resolution by increasing frequency limits the depth of penetration. Although this effect has a potential impact on transducer selection, it has minimal impact on imaging rats and mice because most of the target organs are generally within the penetration depth range (0.5-2 cm) of the higher frequency transducers. In larger species, this relation does influence transducer selection. A higher frequency transducer that could be used to image small nonhuman primates, such as marmosets, may not have the depth of penetration to image pigs or adult beagles.

Temporal resolution, or the ability to distinguish two events in time, is an important consideration when imaging rat and mouse hearts due to their fast heart rate (400-600 beats/min for the mouse, 300-500 beats/min for the rat). Temporal resolution is determined by the number of image frames that can be acquired per second, generally expressed in Hertz (Hz) or cycles per second. The importance of temporal resolution becomes evident when the number of images acquired per cardiac cycle is evaluated. For example, at a heart rate of 300 beats/min, an acquisition frame rate of 30 Hz (30 image frames/sec) would result in only six images/cardiac cycle. Moreover, each image frame would be acquired over 17% of the cardiac cycle, making the accurate determination of absolute end diastole and end systole difficult. In contrast, imaging a mouse heart beating at up to 600 beats/min with a frame rate of 150 Hz would acquire 15 frames over one cardiac cycle, greatly enhancing the temporal resolution.

Several commercially available ultrasound systems can acquire at frame rates of 120 to 600 Hz. Although UBM offers greater spatial resolution, currently available systems have low imaging frame rates (5-10 Hz) and relatively poor temporal resolution for cardiac structures. The temporal resolution is not as critical when imaging target organs that are relatively nonmoving, such as the kidney^[40].

Detection of Fetus by Sonography

Sonography was performed using sonography scanner (AGROSCAN, Manufactured by ECM Portable Ultrasound Producer, France) and was carried out under light ether anesthesia using anesthetic chamber. After anesthesia confirmed in rats, ultrasound gel PHOTOCHEM Ultrasound Gel (Photochem Laboratories Pvt. Ltd. Ahmedabad, Gujarat, India) was applied on the abdomen of the rats. The ALR-500 probe, frequency 5MHz was kept on the abdomen of the rats on the left and right side of the abdomen and automatic mode was

selected. The uteri were examined with same calibration in all areas and trained with the same level of accuracy to obtain the images. The total numbers of implantation sites were determined by observing Sonography markers, including embryonic development; placenta echogenicity, fetal skeleton, fetal heart pulsation, and fetal abdominal viscera. During the whole process animals were remained totally relaxed in calm during whole examination.

Pharmacological Studies

Abortifacient Activity

Rats at day 1 of pregnancy were divided into three groups, consisting of six animals in each group. The first group served as control and received vehicle only (distilled water) and group 2, 3 and 4 received suspension of drugs and poly herbal formulations twice a day respectively from day 10 to 18 of pregnancy. On 19th day of pregnancy, the upper abdomen hairs of rats were removed by shaving using sterile blade. The animals were transported at the Government veterinary hospital, Pedak road, Rajkot by AC Car for Sonography. The uteri were examined to determine the number of implantation sites. The rats were allowed to recover and were allowed to give birth.

RESULTS

Evaluation of Abortifacient Activity

The evaluation of Abortifacient activity was carried out by a dose dependent inhibition of implantation was observed using sonography. The number of fetus remained in each drug treatment group were counted and compared with normal control group. The percentage Abortifacient activity was calculated by comparing the drug treatment groups with normal control group and percentage was calculated. The results revealed that there was a significant decrease in fetus count ($p < 0.01$) in Mifepristone treated drug treatment group (0.16 ± 0.16) as compare to Product-A treated group (6 ± 1.34), Product-B treated group (6 ± 1.34) and normal control group (6 ± 1.34). The percentage Abortifacient activity was found to be 97.22%, 0.0% and 0.0% in Mifepristone group, Product-A group and Product-B group respectively.

Abortifacient activity

Table-1 Grouping and Dosing in Animals

Group	Drug	Dose	Route of administration
Group 1 (Control Group)	Distilled water	1ml twice a day	Oral
Group 2 (Standard)	Mifepristone suspension(3.3mg/kg)	1ml twice a day	Oral
Group 3 (Test-1)	Product-A powdered suspension(8.3mg/kg)	1ml twice a day	Oral
Group 4 (Test-2)	Product-B powdered suspension (5.0mg/kg)	1ml twice a day	Oral

Table- 2 Abortifacient activity in Female Wistar rats

Group	Drug	No of fetus remained	Percentage Abortifacient activity
1	Distilled water	6±1.34	0%
2	Mifepristone powder suspension(3.3mg/kg)	0.16±0.16**	97.22%
3	Product-A powdered suspension (8.3mg/kg)	6±1.34	0%
4	Product-B powdered suspension (5.0mg/kg)	6±1.34	0%

- Values are expressed as Mean ± S.E.M
- **- significantly different from normal control (p < 0.01)
- Group-1=Normal control Group (Distilled water)
- Group-2=Standard drug treatment Group (Mifepristone 3.3mg/kg, 1ml twice a day p.o.)
- Group-3= polyherbal formulation Group (Product-A 8.3mg/kg, 1ml twice a day p.o.)
- Group-4= polyherbal formulation Group (Product-B 5mg/kg, 1ml twice a day p.o.)

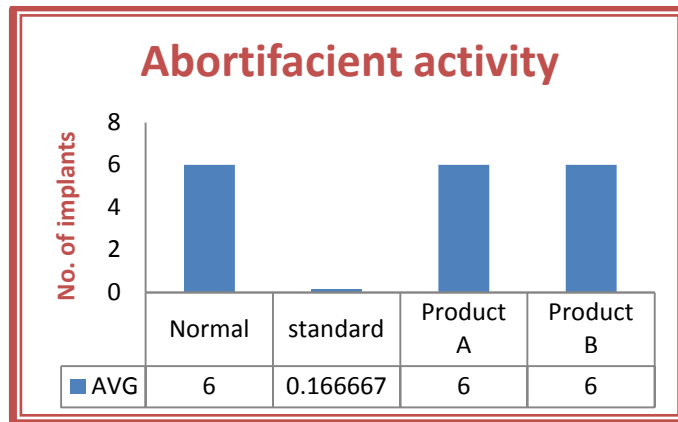


Figure-1: Percentage post-coital Anti-implantation activity

- Values are expressed as Mean ± S.E.M
- **- significantly different from normal control (p < 0.01)
- Group-1=Normal control Group (Distilled water)
- Group-2=Standard drug treatment Group (Mifepristone 3.3mg/kg, 1ml twice a day p.o.)
- Group-3= polyherbal formulation Group (Product-A 8.3mg/kg, 1ml twice a day p.o.)
- Group-4= polyherbal formulation Group (Product-B 5mg/kg, 1ml twice a day p.o.)

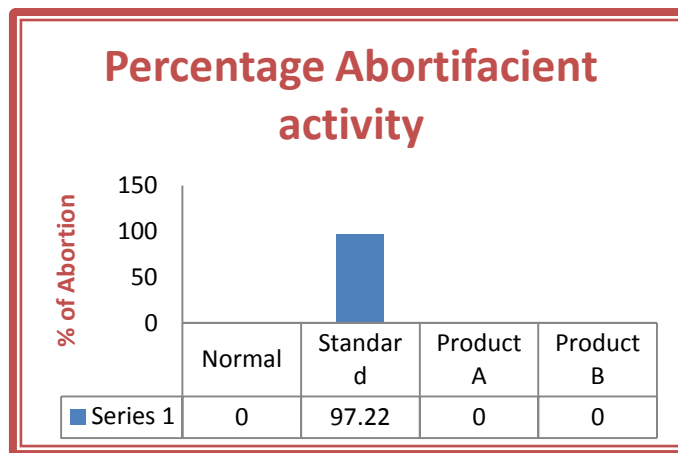


Figure-2: Percentage post-coital Anti-implantation activity

- **- significantly different from normal control (p < 0.01)
- Group-1=Normal control Group (Distilled water)
- Group-2=Standard drug treatment Group (Mifepristone 3.3mg/kg, 1ml twice a day p.o.)
- Group-3= polyherbal formulation Group (Product-A 8.3mg/kg, 1ml twice a day p.o.)
- Group-4= polyherbal formulation Group (Product-B 5mg/kg, 1ml twice a day p.o.)

DISCUSSION

The population growth rate of India in past several years is significantly increased which caused many serious socio-economic problems. There is increasing pollution of water, air and land. Due to the limited natural resources available in our country, there is a shortage of petroleum, minerals and water.

To prevent the birth rate there is an urgent need of antifertility agents. There are many contraceptives available for control of birth but the most commonly used contraceptives are oral contraceptives. All the synthetic oral contraceptives are having common side effects of insomnia, headache, dizziness, breast tenderness, depression, painful uterine contraction. The chronic use of oral contraceptives causes anemia, cancer, diabetes, epilepsy, cardiac disease and thrombosis.

Due to side effects of oral contraceptives, there is increasing popularity of herbal contraceptives due to their less side effects, easy availability and cheapness. The herbs used alone as a contraceptive have no significant effectiveness that it can be used alone as the contraceptive.

The combination of herbs may have the significant antifertility activity as compare to the synthetic steroidal oral contraceptives. In this research work, the polyherbal formulations which are most prescribed by the physicians were selected for the abortifacient activity study. Two marketed polyherbal formulations were selected for study, Product-A and Product-B. They are being used currently as the menstrual cycle regulating drugs. They have the herbal constituents that can have significant antifertility activity. The polyherbal formulation in suspension form were evaluated and compared with the standard Mifepristone synthetic allopathic drug which is commonly used in the allopathic formulations.

The polyherbal formulations and Mifepristone active pharmaceutical ingredient was suspended in the carboxyl methyl cellulose and given by oral route to the female Wistar rats. The dose was selected and fixed on the average body weight of the rat (250-300 mg.) comparing with human adult dose of the polyherbal formulations in respect of average female body weight (60 kg.). The dose was given twice a day by oral route which is directed by many physicians to the patients.

There was mainly one type of pharmacological studies on antifertility activity. The antifertility activity was evaluated using Abortifacient activity.

There was no significant Abortifacient activity seen in

polyherbal formulation like Product-A and Product-B as a highly used market formulations compare to standard Mifepristone drug. The percentage abortifacient activity was found to be more potent in standard Mifepristone drug. The percentage abortifacient activity in Mifepristone was seen 97.22% compare to normal, Product-A and Product-B were 0.0%.

From the results we concluded that the polyherbal formulations are no significant effective in normal dose to produce the antifertility activity.

SUMMARY AND CONCLUSION

From our Research, following important inference reveals –

We have confirmed that in normal dose (Market product-A and Product-B) there is a no abortifacient activity seen in Polyherbal formulations in comparison of standard Mifepristone drug.

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