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DETERMINATION OF SPERMICIDAL ACTIVITY OF PLANT EXTRACT ON GOAT SEMEN

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ABSTRACT

Fertility control is an issue of global & national public health concern. The traditional use of medicinal plants to treat different sorts of disease, including anti-fertility, is widespread throughout the world as many plant substances are known for their interference with male reproductive system. Approximately 50% of all pregnancies are unintended at conception; 50% of those occur in the 94% of sexually active couples who report using some method of contraception. The only male-specific contraceptive methods currently available are withdrawal, condoms and vasectomy. As concerns regarding side effects and inconvenience of the existing methods of contraception (both female and male) prevent their universal acceptance, the development of additional male methods of fertility control can provide tremendous social and public health benefits. Tests on goat semen were done using *Mentha arvensis* & *Momordica charantia*. Mass activity and Initial Motility assays as well as live and dead percentages of number of sperms in the semen sample were determined. Hypo-osmotic swelling test was also carried out. It was observed that the plant extracts change the plasma membrane integrity of the sperms and lead to their

death. Thus, the plant extracts show spermicidal activity. Further research should be carried out to develop herbal contraceptives from *Mentha arvensis Linn.* and *Momordica charantia.*

KEYWORDS: Spermicidal activity, Mass Activity, Motility, Hypo-osmotic, Herbal contraceptives.

INTRODUCTION

Rising human population throughout the world, more particularly in developing and underdeveloped parts, has detrimental effects on the life supporting system on the earth. The possibility of an effective check on human fertility may soon be realized through biological means. Fertility regulation comprises of contraception and management of infertility forms and important component of reproductive health. Though considerable progress has been made in the development of highly effective, acceptable and reversible methods of contraception among females, progress and possibilities on males are still slow and limited. With recent progress towards a better understanding of male reproductive physiology there is a need to develop new contraceptive modalities for males.[1]

Several potential approaches for induction of infertility have been investigated over a long period including hormonal chemical and immunological approaches. The chemical compounds affecting testicular functions include different groups like steroidal and non-steroidal. Among them are Danazol deport Medroxy Progesterone Acetate (DMPA), cyproterone acetate (CPA), levenogestral, melatonine, α chloro-hydrene, metapiron and cerotonine. But all of these compounds have been seriously questioned owing to the various hazards as they were proved toxic or idiosyncratic on both the short as well as long term use in the reproductive organs. Despite the availability of various contraceptive modalities one of the most challenging pursuits in the realm of pharmaceutical and medical sciences is the search for newer, more potent, additionally safe and less expensive method that require infrequent and self-administration and should have long lasting but complete reversible anti-fertility effects.[2]

Fertility control is an issue of global and national public health concern. Many studies have been done on male contraception. The traditional use of medicinal plants to treat different sorts of diseases including fertility related problems is widespread throughout the world as many plant substances are known for their interferences with the male reproductive system. Recently, efforts are being made to explore the hidden wealth of medicinal plants for

contraceptive use. With the exciting prospects of gene therapy, herbal medicine remains one of the common forms of therapy available to much of world's population to maintain health and to treat diseases.

There has been a steady accumulation of information regarding the screening of plants having anti-fertility efficacy. The folklore information and ancient literature about plants and herbs can help the anti-fertility programs. In the recent past, a number of plants have been identified and evaluation of extracts and active components of different plants like seeds, roots, leaves, flowers, stem or stem barks have been done by various researchers.[3]

Many ethno botanical surveys on medicinal plants used by the locals have been performed in different parts of the world. Several plant species have been described as anti-fertility agents. The practice of traditional medicine for the control of fertility in most part of Ethiopia and India is based on the use of plant medicines for many years. Several medicinal plants have been used as dietary adjuncts and in the treatment of numerous diseases including for inducing infertility without proper knowledge of that function. Although different herbal plants possess different anti-fertility activities such as anti-implantation, abortification, ecbolic, estrogenic and spermicidal, a large number of medicinal plants possess some degree of toxicity.[4]

Each year around 18 million people are adding to our total population. This imposes an extra burden on the community and it is also the leading cause of poverty and pollution in developing countries. For this purpose, World Health Organization (WHO) has constituted a population control program, which includes studies having traditional medical practices. From the advancement of reproductive biomedicine, several hormonal contraceptive pills have been developed but none are free from side effects. At present, global attempts are being taken to search out the effect of herbal product for contraceptive purposes. Few herbal contraceptives have been developed but the potentiality of these contraceptives is very minimal and the mode of action is beyond of our knowledge till now. Epidemiological studies indicate that combined oral contraceptives increase cerebral thrombosis, increased serum level of triglyceride, HDL and cholesterol and increase family mortality due to cardio vascular diseases as well as malignant tumors in any organ, poor glucose tolerance or diabetes or nausea, abdominal pain, headache, obesity and menstrual changes.

More than 35,000 plant species are being used in different human cultures around the world for medicinal purposes. Nearly 80 % of human population relies on traditional medicines for primary health care, most of which use plant extracts. The status of herbal medicine has been first all over the world during the last few decades. The World Health Organization has set up a task force on plant research fertility regulation with an objective to find new orally active anti-fertility medicines. In India, phytotherapy has a very long tradition although proper scientific explanation is relatively new. In our country as well as in the world there are several medicinal plants associated with anti-fertility properties. Although very few contraceptives have been developed from plant extracts their potentiality has not been determined properly and their mode of action has been beyond our knowledge. Until now, there have been many problems accessing plant extracts including batch to batch variation and the lack of definite active portion of the extract used for the development of herbal contraceptives.[5]

Herbal 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 a very high population like in India, China and Bangladesh. Studying the potency and toxicity of local plants that are reputed for birth control in the folkloric medicine of these countries may generate a greater confidence in and wider acceptance of herbal contraceptives. However, the search for an orally active safe and effective plant preparation and its active compound is yet to be needed for fertility regulation due to incomplete inhibition of fertility or side effects.

Numerous herbs have been used historically to reduce fertility and modern scientific research has confirmed the anti-fertility effects in at least some of the herbs tested. Herbal contraception may never reach the level of contraceptive protection as the pill but it offers alternative for women who have difficulty with modern contraceptives options. There are many different ways the herbs can impair fertility. Some herbs may affect the ovary while others act upon the uterus, affect spermicidal activity or affect normal production or block certain hormones. There are also some herbs that have been found to interfere with normal sperm production or mobility. Some herbs have the ability to interfere with implantation. Those herbs can be used on the basis of need and can be used as emergency purpose.

For century's herbal preparation have been connected with the goal of preventing and or disrupting pregnancy. Scientific researchers have done some research on anti-fertility agents

some findings have been very interesting and promising. Herbal medicines should be one of the common forms of therapy available to much of world's population having anti-fertility effects.

Spermicides are a biological obvious way of interfering with fertility and have an advantage that they don't depend upon high skilled people for their prescription and use. Spermicidal agents are referred as drugs that have ability to immobilize or kill the sperm upon contact. An ideal spermicide should immediately and irreversibly produce immobilization of the sperm, should be non-irritating to the vaginal and penile mucosa, should not have adverse effects on the developing fetus, should be free from long term topical and systematic toxicity and should not be systematically absorbed. Understanding the morphology of spermatozoa is essential to appreciate the mechanism of action of spermicide.

MATERIAL AND METHODS

Procurement of raw plant material and extraction

Source of plant

Leaves of Wild Mint (*Mentha arvensis)* and seeds of Bitter Gourd (*Momordica charantia*) were collected freshly from the place where they are cultivated. These two plants are easily available in the market at a low market price & have various medicinal uses, hence these plants were chosen. The leaves and seeds were washed, shade-dried and powdered and then used for extraction.

Cold Extraction

The powdered leaves were weighed and 30 gms was dissolved in 100 ml of Methanol. The solution was then incubated at room temperature for 24 hrs. The solvent was then filtered and the filtrate was kept in the freezer. The remaining powder was then again dissolved in 100 ml Methanol and this process was repeated 3-4 times. Once the final filtrate was obtained, they were extracted through Rotary Evaporator at 30-35°C. The extract obtained was used for further analysis.

Collection of Semen

Artificial Vagina

The artificial vagina consists of- a) An outer heavy rubber cylinder, b) Inner sleeves of rubber, c) Semen receiving cone and d) Semen collecting vial made of glass. Prior to collection, all these parts are cleaned, sterilized and assembled into artificial vagina.

To prepare the artificial vagina, first put the inner sleeve/liner into the outer cylinder so that both the ends of the inner sleeve are reflected. This forms a water tight space between the two and then water at 40-50°C is filled in along with air to maintain the pressure. Then the cone attached with the collecting vial is slipped through one of the ends and secured with the help of rubber bands following which it is invaginated into the artificial vagina and lubricated with sterilized jelly (Figure 1-4).

Figure 1: Collection cups.

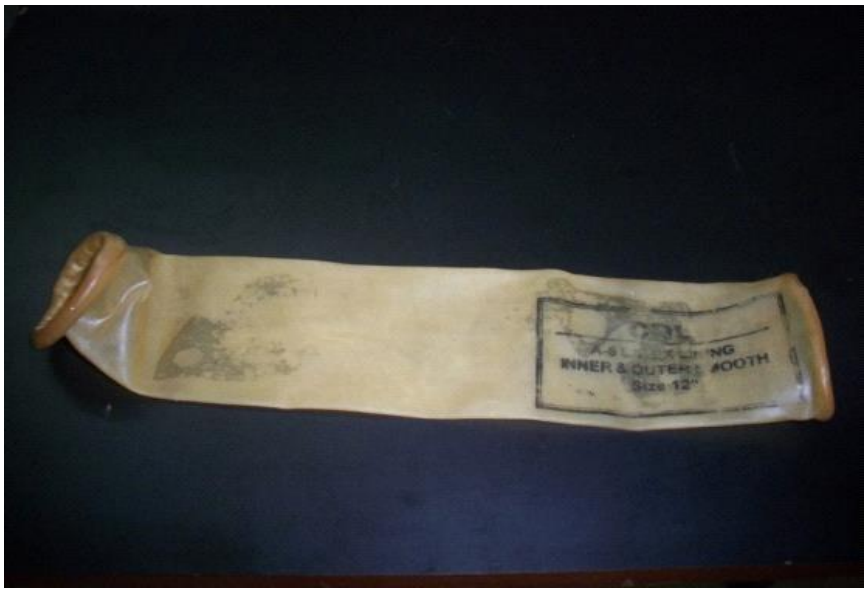

Figure 2: Liner.

Figure 3: Artificial vagina.

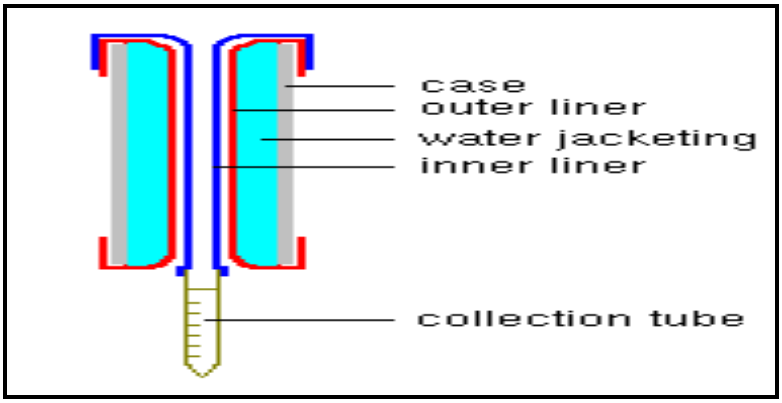

Figure 4: Artificial vagina.

Semen Collection Procedure

A young buck is encouraged to mount on another buck or an anestrus doe and if it refuses initially, then heat can be used for collection. The artificial vagina is prepared and attached to the buck's penis when it is attempting to push and guide the doe/buck. The sperm is then collected in collection cups and used for further study.

Characteristics of Semen

Color

The color of goat semen may be white, creamy or slightly yellowish.

Consistency

The consistency varies from thin to thick depending upon concentration of spermatozoa.

Volume

The ejaculate volume varies from 0.25-5 ml but is usually between 1.5-2 ml. Higher ejaculate volume may be associated with poor quality of semen.

pH

It varies from 6.5-7.0 which means it is alkaline.

Morphological Characteristics

The head of the spermatozoa have a mean length and width of 8.39 µm and 4.37 µm respectively, and the mean width of the base of the sperm head is 2.45 µm. Length and width of middle piece were 11.81 µm and 1.06 µm. Length of the tail was 41.26 µm. Ribonucleic acid is not found in mature spermatozoa, but it is found in spermatogonia & spermatocyte cells. The lipid content in spermatozoa is mostly plasminogen. The enzyme hyaluronidase appears to be contained in the head region of spermatozoa. In the mid-piece of spermatozoa, the enzymes responsible for the aerobic and anaerobic metabolism as well as for the motility and respiration of spermatozoa are located.

Biochemical Characteristics

Goat spermatozoa contain Serum Glutamic Oxaloacetic acid Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT). These enzymes are present in seminal plasma as well as in mitochondria. There are other enzymes like alkaline phosphatase, lactate dehydrogenase, etc. which are also present in the seminal plasma as well as spermatozoa. Fructose is the normal sugar providing a source of energy for spermatozoa in the semen. It is produced in the seminal vesicles. Citric acid is also present in semen. Other substances are sodium, phosphorous, inositol, sorbitol and glycerol phospholinecholine. Goat semen contains a peculiar “egg yolk coagulating enzyme” which is said to be secreted in the Cowper’s glands.

Evaluation of Semen Sample

Mass Activity

Mass activity, also referred to as gross motility, is the wave motion or swirl visible when a sample is viewed under low power on a microscope slide. For an effective result it is important that the sample is not subjected to a cold shock (e.g., placing the semen sample onto a cold microscope slide) or has urine contamination as both will significantly reduce sperm motility. Mass activity is influenced by the age of goat, collection method and the age of the semen. Satisfactory semen should have a mass activity score of 3 or better.

First the semen was collected in the collection cup by the help of an Artificial Vagina. The plant extract was mixed with the semen sample in a 1:1 ratio. Then immediately on a slide

one drop of semen sample was placed along with the test material. It was observed under light microscope with 10X objective without a cover slip.

Determination of Initial Motility

In order to achieve fertilization, a sperm must not only be able to move but also be capable of movement that results in forward progression. For this, fresh semen sample was diluted with Phosphate Buffered Saline (PBS) in a ratio of 1:4 or 1:8 in an Eppendorf tube prior to examination. The test sample was then added to the diluted semen sample and then 20 µL of it was placed on a Makler Counting Chamber and covered with a special cover slide. It was examined under 10X magnification and only the motile sperms, in forward progression, were counted within ten boxes and recorded. The same boxes were recounted, this time only counting the non-motile sperm.

Sperm Viability

The percentage of live sperm in a sample can be estimated by viewing under a microscope. Dead sperm do not necessarily indicate a problem. They may simply be the result of normal attrition when a goat is not actively working and the result must be interpreted in conjunction with other traits and the goat's activity. Repeat testing can be done if of concern.

Sperm vitality is reflected in the proportion of spermatozoa that are "alive". The eosin-nigrosine staining technique is based on the principle that dead cells will take up the eosin and as a result stain red. The nigrosine provides a dark background, which makes it easier to assess the slides. The unstained spermatozoa with transparent head & tail are counted as live and pinkish eosinophilic or total or partial stained spermatozoa are classified as dead. Eosin is usually a fluorescent red dye which is a result of bromine. The dye is acidic and acts on the basic parts of cells such as collagen, cytoplasm, muscle fiber, etc. It mainly binds to protein. Nigrosine is used for negative staining and it is an acidic dye.

50 µL of semen was mixed with 2 drops of stain A (20 ml of 1% Eosin in water) in a sterile test-tube. After 30 seconds, three drops of stain B (30 ml of 10% Nigrosine in water) were added and mixed thoroughly. Within 30 seconds of adding stain B, a drop of semen-stain mixture was placed on a microscope slide and a thin smear was made using a cover glass. It was allowed to air-dry and then examined under oil immersion (100X magnification) under bright field microscope. Colorless spermatozoa were considered alive and red stained spermatozoa were considered dead. Up to 100 cells were counted.

Hypo-osmotic Swelling Test

The hypo-osmotic swelling (HOS) test enables the identification of sperm with functionally intact membranes and is one of a range of tests commonly used to determine sperm viability. The osmotic stress caused by the chosen hypo-osmotic medium must be sufficient to affect an influx of water into the cell to result in an increase in volume and hence curling of the tail, but to prevent lysis of the sperm membrane. The HOS test is an ideal method being quick, simple and requiring minimal equipment, and has been shown to correlate well with supravital staining. The HOS test should not be used as a sperm function test but may be used as an optional, additional vitality test. It is simple to perform and easy to score and gives additional information on the integrity and compliance of the cell membrane of the sperm tail. The HOS test may help in assessing the diagnosis and the management of male infertility.

Two hypo-osmotic solutions were prepared viz. 1.351 gms of fructose in 50 ml distilled water and 0.735 gms sodium citrate in 50 ml distilled water. Both these solutions were mixed in an equal ratio (0.5 mL each) and kept in the incubator at 37°C for 10 mins. Then 50 µL of the semen sample was added in this solution and incubated at 37°C for 30 mins. 10 µL of the incubated sample was taken on a glass slide, covered with glass cover and observed under 10X objective lens.

RESULTS

Plant Extract

Cold methanol extraction was performed to obtain the extracts of plants. The weight of extracts was found to be 7.3106 gm and 5.780 gm for *Mentha arvensis* leaves and *Momordica charantia* seeds respectively. After obtaining all the extracts they were stored at 4ºC for further use.

Mass Activity

Table 1 shows that the time required for the complete loss of mass activity for 5 mg of *Momordica charantia* extract and 5 mg of *Mentha arvensis* extracts show good results as compared to control.

Table 1: Mass activity of semen sample.

No. of goats	Time required for complete loss of mass activity (mins)				
	Control	*M. arvensis*		*M. charantia*	
		1 mg dose	5 mg dose	1 mg dose	5 mg dose
1	20	8	4	6	3
2	19	7	5	6	4
3	19	8	4	6	3
4	18	8	4	7	3
5	20	8	4	7	3
6	20	8	4	6	4
7	19	7	5	7	4
8	18	7	4	7	3
9	20	7	4	6	3
10	20	8	5	6	3
Average	**19.300** [a]	**7.600** [b]	**3.900** [c]	**6.100** [d]	**3.400** [e]
F value	**300.850***				

** Significant at 5 % level. Means with common superscripts do not differ significantly C.D. (0.05 = 1.070)*

From Figure 5, it is seen that after putting the extracts to the semen sample the mass activity was drastically changed. 5 mg dosage of both extracts showed better results as compared to control as well as 1 mg dosage. It was also observed that 1 mg of *Momordica charantia* extract showed good result as compared to 1 mg *Mentha arvensis* extract, same results were obtained in case of 5 mg extract also in respect of time. Afterwards, it was observed that 5 mg *Momordica charantia* extract show the most significant results among all other dosages.

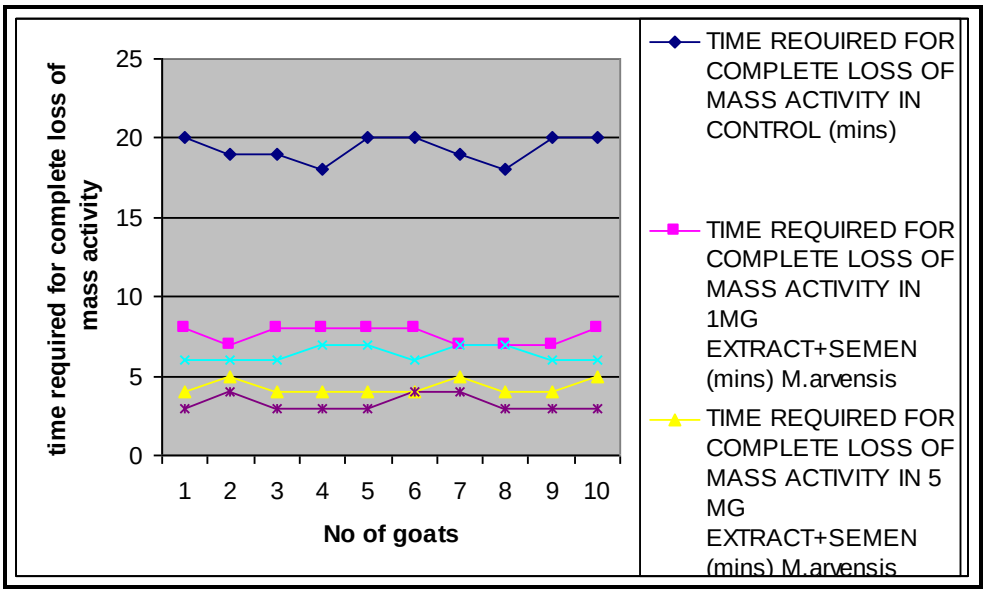

Figure 5: Comparative study of mass activity of semen sample for both extracts in respect to time & dosage.

Initial Motility

It was observed that after dilution of semen sample the initial progressive movement of single spermatozoa lasted for a long time whereas after mixing the plant extract it rapidly changed and within a very short period of time no progressive movement was seen. It was seen that 5 mg dosage gave better results when compared to control as well as 1 mg dosage. In 5 mg dosage within a very short time period progressive forward movement of single spermatozoa was not found in both *Mentha avernsis* and *Momordica charantia* extracts (Refer Table 2). From Figure 6, it was obtained that individual forward motion of spermatozoa was lost after treating the semen sample with the extracts. 5 mg of both the extracts show good result as compared to the control as well as 1mg extract.

Table 2: Result for Initial Motility semen sample,

Goat no.	Time required for complete loss of motility (mins)				
	Control	*M. arvensis*		*M. charantia*	
		1 mg dose	5 mg dose	1 mg dose	5 mg dose
1	75	10	7	9	6
2	80	11	7	9	5
3	75	11	8	8	6
4	75	10	8	8	5
5	80	11	7	9	5
6	80	10	9	8	6
7	75	10	8	9	6
8	75	10	7	8	5
9	80	10	7	8	5
10	75	10	8	9	6
Average	**77.00** [a]	**10.30** [b]	**7.60** [c]	**8.50** [d]	**5.50** [e]
F value	**5920.777***				

**Significant at 5 % level. Means with common superscripts do not differ significantly C.D. (0.05 = 1.153)*

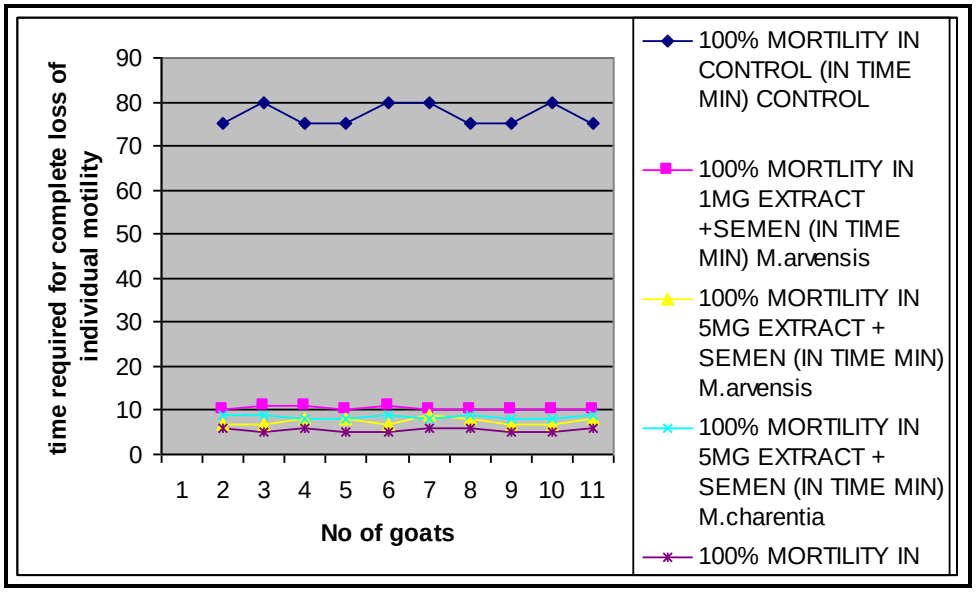

Figure 6: Comparative study of initial motility of both samples in respect to time & dosage.

Percentage of Live cells

From Table 3, it has been concluded that the live cell percentage decreased after giving treatment of 5 mg and 1 mg dosage of the plant extracts. Percentage of live cells was decreased to 4-10 % in 5 mg dosage and 12-25% in 1 mg dosage.

Table 3: Percentage of live & dead spermatozoa treated with *Mentha avernsis* extract.

Number of goats	Control	*Mentha arvensis*		*Momordica charantia*	
		1 mg dose	5 mg dose	1 mg dose	5 mg dose
I	90	25	9	15	4
2	91	20	10	14	5
3	92	20	9	14	4
4	88	15	7	12	4
5	92	17	7	15	5
6	90	15	9	12	7
7	91	20	10	15	6
8	92	15	7	12	6
9	90	17	8	14	5
10	88	15	10	14	4
Average (live)	**90.40** [a]	**17.90** [b]	**8.50** [c]	**13.60** [d]	**5.30**[e]
***F value**	**4069.46**				

**Significant at 5 % level. Means with common superscripts do not differ significantly C.D. (0.05 = 1.605)*

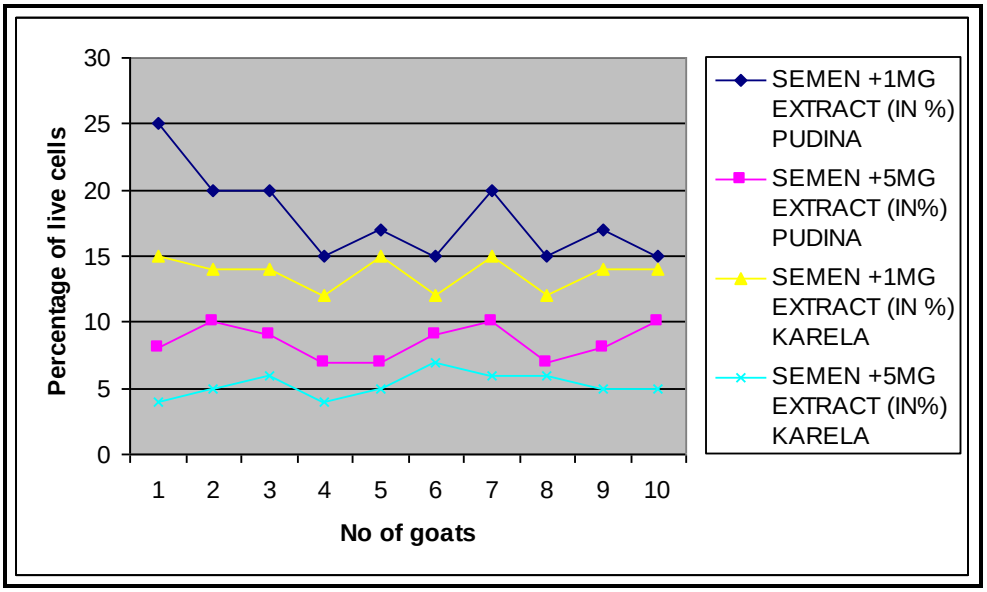

Figure 7: Comparative study for the percentage of live cells for both samples.

From Figure 7, percentage of live cells was drastically decreased after the treatment of both plant extracts. As like other data, it also revealed the best significant result which was shown by 5 mg concentration of both the extracts as compared to the control and as well as 1 mg concentration.

In Figure 8, all the sperms having pinkish color are dead and sperms having transparent head and tail considered to be live.

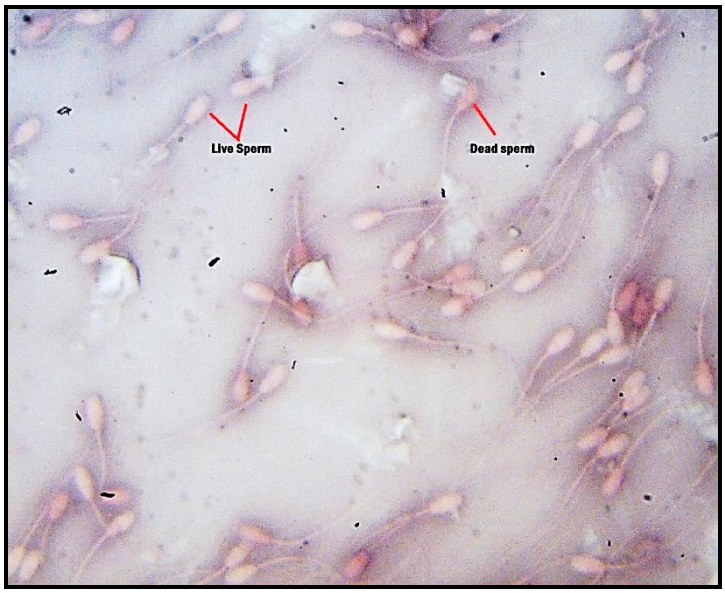

Figure 8: Live & Dead spermatozoa- Eosin–Nigrosine staining.

Result of Hypo-osmotic swelling Test (HOS)

Table 4: Result of percentage HOS positive cells in semen

Goat no.	Control	*Mentha arvensis*		*Momordica charantia*	
		1 mg dose	5 mg dose	1 mg dose	5 mg dose
1	60%	10%	6%	4%	2%
2	65%	15%	5%	5%	2%
3	62%	10%	5%	5%	3%
4	60%	14%	6%	6%	NIL
5	65%	15%	5%	4%	NIL
6	64%	11%	5%	5%	2%
7	60%	13%	5%	4%	3%
8	65%	10%	6%	5%	NIL
9	63%	12%	4%	4%	2%
10	64%	11%	5%	5%	NIL
Average	**62.80** [a]	**12.10** [b]	**5.20** [c]	**4.70** [d]	**2.10** [e]
*** F value**	**3268.231**				

**Significant at 5% level. Means with common superscripts do not differ significantly C.D. (0.05 = 1.282)*

From Table 4, it was revealed that in control sample the HOS positive cell percentage is significantly higher than the semen treated with 1 mg and 5 mg dosage of plant extracts**.**

From Figure 9, it was observed that plasma membrane integrity was almost completely lost in case of treatment of semen with 5 mg concentration of each extract, whereas in control it showed a wide range of HOS positive spermatozoa that is with intact plasma membrane integrity. 1 mg concentration of extracts also showed good results as compared to control.

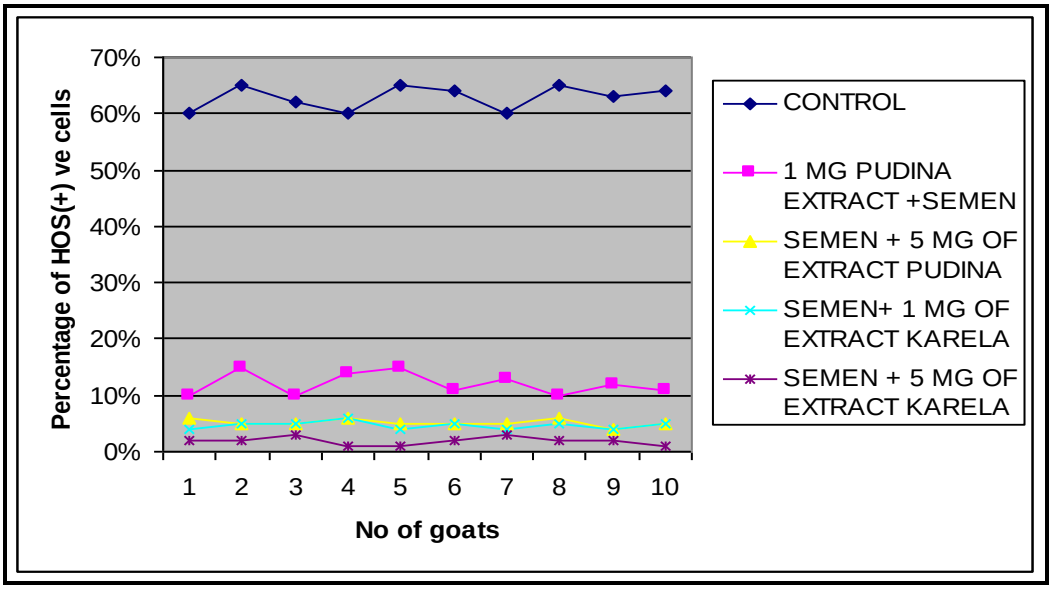

Figure 9: Comparative study for HOS positive spermatozoa for both extracts.

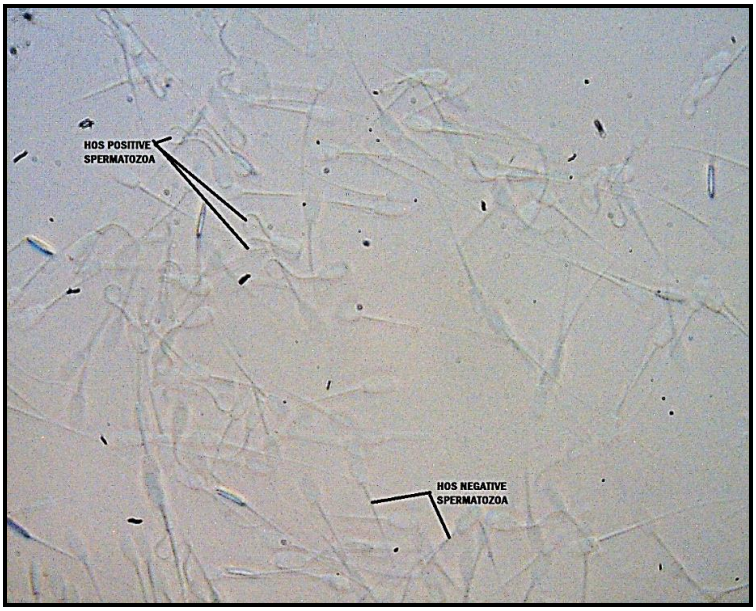

Figure 10: Hypo-osmotic swelling test.

DISCUSSION

Population control measures by the way of contraceptive have remained a practice since antiquity. Human population control is the practice of artificially altering the rate of growth of a human population. Historically, human population control has been implemented by limiting the population's birth rate, usually by government mandate and has been undertaken as a response to factors including high or increasing levels of poverty, environmental concerns, religious reasons and overpopulation. While population control can involve measures that improve people's lives by giving them greater control of their reproduction, some programs have exposed them to exploitation.[1]

Worldwide, the population control movement was active throughout the 1960s and 1970s, driving many reproductive health and family planning programs. In the 1980s, tension grew between population control advocates and women's health activists who advanced women's reproductive rights as part of a human rights-based approach.[2] Growing opposition to the narrow population control focus led to a significant change in population control policies in the early 1990s.

Various physical, physiological and chemical methods have been used to design contraceptive that are effective and are yet non-toxic. Another solution proposed by this group is the use of quinacrine, a sterilization drug for women. Quinacrine is only one of the ways of sterilization. There is also surgery, and of course, the ever-present birth control pill.

But quinacrine acts in a different way. Although it has not yet been approved for testing in most places, it is a procedure that is gaining favor with certain population control advocates. The procedure itself is called quinacrine sterilization. Quinacrine are chemical pellets that are introduced into the uterus via a modified intrauterine device applicator. They are placed about ½ of a centimeter from the fundus. The pellets then dissolve, filling the upper womb and fallopian tubes with quinacrine hydrochloride. Quinacrine hydrochloride is a very strong acid. The acid leaves severe chemical burns in this sensitive tissue, which in turn, leaves scarring, thus effectively blocking the fallopian tubes and preventing conception.

About 40% of women who take birth-control pills will have side effects of one kind or another during the first three months of use. The vast majority of women have only minor, transient undesired effects. Some side effects are uncommon but may be dangerous. The chances of birth control pills contributing to a heart attack are present. Women taking birth control pills usually have a small increase in both systolic and diastolic blood pressure, although readings usually remain within the normal range. Women who take oral contraceptive and have a history of migraines have an increased risk of stroke compared to non-users with a history of migraine.[4] Women who use birth control pills are at a slightly increased risk of having blood clot in the legs or lungs. This side effect usually goes away after the first few months of use or can be prevented by taking the pill with a meal. Anything that makes the pill go through your system too fast can make the pill not work as well because it was not absorbed or, worse, if it is lost in the vomit.

The pill costs more than some other methods of contraception. One of the major methods of achieving in the present-day times is using spermicidal agents. Although a number of spermicidal contraceptive agents are available in the market, their toxicities have been found with their wide spread usage. Many herbal preparations have been reported in traditional medicine as potential spermicidal agent.

Two such sources are *Mentha arvensis* and *Momordica charantia*. The present study was designed to evaluate the spermicidal activities of extracts of *Mentha arvensis* and *Momordica charantia*. Cold methanol extracts of the plants were prepared. The extracts were then subjected to different tests like- a) Mass activity, b) Initial motility c) Live & dead cell counting and d) Hypo osmotic swelling test.

Reduction in sperm mass activity was observed after addition of plant extracts into the semen and it was dose and time dependent. The average time taken to achieve 100 % mortality was around 5 mins and 8 mins at dose of 1 mg/ml and 5 mg/ml respectively for *Mentha arvensis*, whereas the same for *Momordica charantia* extract was found to be at 6 mins and 4 mins for 1 mg/ml and 5 mg/ml respectively and for control it was found to be 20 minutes.

There also occurred reduction in initial sperm mortality after addition of plant extracts. It was observed that the extracts of *Mentha arvensis* were able to reduce the initial mobility of sperm by 86.67 % at the dose of 1 mg/ml in 8 mins and around 90.97 % at the dose of 5 mg/ml in 4 mins and the extracts of *Momordica charantia* were able to reduce the initial mobility of sperm by 83.45 % in 7 mins at the dose of 1 mg/ml and around 92.54 % in 3 mins at dose of 5 mg/ml.

The extracts of *Mentha arvensis* at 1 mg/ml and 5 mg/ml concentration were able to kill 86 % and 90 % of goat sperms while the extract of *Momordica charantia* at 1 mg/ml and 5 mg/ml concentration were able to kill 83 % and 93 % of goat sperms. At the same time in control, it was found that only 20 % of sperms were killed.

Hypo-osmotic swelling responses reflect integrity of the sperm membrane. The intact sperms permit free passage of the fluid into the cell to reach in the osmotic equilibrium on exposure to osmotic environment, thereby increasing the sperm volume and bulging of plasma membrane.[21][22] The sperms treated with extracts did not show any curling of tail. The semen sample treated with *Mentha arvensis* extract showed only 10 % and 6 % cells were positive at dose of 1 mg/ml and 5 mg/ml respectively while same for *Momordica charantia* was found to be 4 % and 2 % at 1 mg/ml and 5 mg/ml respectively. This observation suggests that the functional integrity of sperm was lost following exposure to extracts.

After statistical data analysis, results revealed that extracts of *M. arvensis* and *M. charantia* presented with good spermicidal activity in a very short period of time as compared to the control. Both of the dilutions that is 1 mg/ ml and 5 mg/ml show better results as compared to control. In case of mass activity, it was observed that there was a drastic change in mass activity after treating the semen with the extracts. In initial motility, the forward progressive movement of individual spermatozoa was completely lost after treating with the plant extract. Afterwards, it was also observed that the percentage of live cells was decreased as compared to control & the plant extract treated sample also show a significant loss of plasma membrane

integrity as compared to control. So, after looking through all these data obtained it could be said that these extracts have good spermicidal activity.

CONCLUSION

After statistical data analysis revealed that these two extracts of *M. arvensis* and *M. charantia* presented good spermicidal activity in a very short period of time as compared to the control. Both of the dilutions that is 1 mg/mL and 5 mg/mL show better results as compared to control. Further studies are required to isolate the active fraction from the crude extract to ascertain the exact mechanism responsible for its spermicidal potential. Further experiments, such as comet assay, can be done to see whether the plant extracts are responsible for DNA damage or not.

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