Effect of *Cinnamomum camphora* on human sperm motility and sperm viability

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Abstract- Sperm motility is a crucial part of examination for studying the effect of any compound. On microscopic examination, sperms look like a swimming tadpole. Each sperm structure is composed of a head which contains the genetic material of father in its nucleus part, a tail which lashes back and forth to propel sperm along, and a midpiece which has mitochondria (power house of sperm) which provide energy for sperm motion. The presences of sugar fructose produced by seminal vesicles provide energy for sperm motility. An alkaline pH of sperm is an important characteristic. Camphor, from the above study is regarded as poisonous if ingested in large doses, it acts as a contraceptive agent, an abortifacient, as suppressor of sperm motility reducing its capacity to fertilize in rats. On other hand, it has several health benefits. In this study of effect of camphor on human sperm motility and its viability, a decrease in sperm motility and sperm viability investigated with increased concentration of camphor solution. It may be due decrease in fructose levels, or denaturation of proteins, cholesterol which are indirectly connected with energy source for sperm motility. Thus, it can be indicated that there is a positive relation between Camphor and sperm parameters. Hence, camphor treated samples shows descent in sperm motility count which counts for decreasing effectiveness for fertilization and thus acts as a contraceptive.

Key words - Sperm motility, camphor, mitochondria and fertilization

Introduction

Today, rapidly expanding population and limited sources are thought to be the most pressing global problems. Fertility control is very essential for maintaining satisfactory standards in the developing countries. The population of India is multiplying at an alarming rate and has almost crossed one billion as per population survey concluded recently. Fertility regulation has therefore become the major concern for the people of all the walks of life. The dramatic success of oral contraceptives in women and the lack of pill for man have stimulated research in male fertility control. The development of new and improved contraception agent for men has lagged behind the development of female contraceptives. The male, an integral part of the family unit, has largely been sidelined by family Planning advisors. Currently, efforts are being made to develop a male contraceptive agent, which would inhibit fertility without affecting sex accessory function. Thus, there is an immediate need for an inexpensive, safe, and effective as well as universally acceptable contraceptive. For evolution of such an ideal method for control of human fertility, it is necessary that the reproductive process both of male and female need to be more intensively investigated [1-6].

Human fertilization is the union of a human egg and sperm, usually occurring in the ampulla (the second portion) of the uterine tube. Spermatozoa, after passage through the epididymis (coiled segment of the spermatic ducts that serves to store, mature and transport spermatozoa between the testis and the vas deferens), are motile cells. Sperm motility becomes critical at the time of fertilization because it allows or at least facilitates passage of the sperm through the zona pellucid. Without technological application, a non-motile or abnormally-motile sperm is not going to fertilize. The evaluation of sperm size, shape and appearance characteristics should be assessed by carefully observing a stained sperm sample under the microscope. The additions of colored “dyes” (stains) to the sperm allow the observer to distinguish important normal (characteristics) as well as abnormal finding. Normal sperm have oval head shapes, an intact central or "mid" section, and an uncoiled, single tail. Several different shapes or forms of human sperm have been identified and characterized. These forms fall into one of four main categories: normal

forms, abnormal head, abnormal tail and immature germ cells (IGC). Many different sperm head abnormalities may be seen. Large heads (macrocephalic), small heads (microcephalic) and an absence of identifiable head are all seen in evaluations. Tapering sperm heads pyriform heads (teardrop shape) and duplicate or double heads have been seen. Overall (gross) abnormalities in appearance may be termed "amorphous" changes. Coiling and bending of the tail are sometimes seen. Broken tails of less than half normal length should be categorized abnormal. Double, triple and quadruple tails are seen and are abnormal.

Sperm Counts

Sperm count is a part of a fertility evaluation. The motile density is the most important part of the semen analysis. This value is essential in both allowing a determination regarding whether or not a semen analysis is "normal", as well as in providing prognostic information for reproductive medical assistance.

Normal counts

Total density or count - (*20 million per ml or above*)

Total motile density- (*8 million per ml or higher*).

Sperm "Motility" (Movement)

Sperm motility is one of the biological characteristics of the spermatozoa. The quality of the sperm is more significant than the count. Motility is graded by the criteria according to the World Health Organization (WHO) Manual as “a” to “d”. This is detailed as below:

1. Grade a (fast progressive) sperms are those which swim forward fast in a straight line - like guided missiles;
2. Grade b (slow progressive) sperms swim forward, but either in a curved or crooked line, or slowly (slow linear or non linear motility);
3. Grade c (no progressive) sperms move their tails, but do not move forward (local motility only);
4. Grade d (immotile) sperms do not move at all.

Sperms of grade c and d are considered poor. Poor motility is called asthenozoospermia which is associated with reduced viability of spermatozoa. It is seen that high percentage of spermatozoa in the sample have less than normal motility. Frequent causes of asthenozoospermia are abnormal spermatogenesis (sperm manufacture), epididymal sperm maturation problems, transport abnormalities, varicocele. Non-motile spermatozoa are called necrozoospermia. It is characterized by total absence of moving sperm. The factors causing necrozoospermia are varied from diet, disease, injury, medications, alcohol or illicit drug use etc. It is has been reported that sperm with normal reproductive genetics are deficient in one or several of the factors necessary to produce motility.

Sperm motility is the number of motile (moving) sperm seen in an ejaculate specimen. Sperm motility is a temperature dependent sperm function, so the handling and processing of specimens is critical. Sperm are known not to survive well for extended periods of time in semen, and in nature, sperm very rapidly leave the semen to enter the cervical mucus.

There are number of studies has been carried out for estimation of the viability of the sperms by staining with Eosin stain and percent viability is calculated by using standard formula. Non motile sperms are tested for its viability by the mixing fresh semen with a supra-vital dye such as eosin or trypan blue, or by the use of the hypo osmotic swelling test (HOS), based on the semi-permeability of the intact cell membrane, which causes spermatozoa to “swell” under hypo-osmotic conditions, when an influx of water

results in an expansion of cell volume (Drevius & Eriksson, 1966). Jeyendran et al. (1984) introduced above mentioned test for Non-motile viable sperm and suggested that Non-motile viable sperm for Intra cytoplasm sperm injection (ICSI) may be used for *in vitro* fertilization procedure in which a single sperm is injected directly into an egg.

Cinnamomum camphora (commonly known as Camphor tree, Camphorwood or camphor laurel) is a large evergreen tree that grows up to 20–30 metres tall. The leaves have a glossy, waxy appearance and smell of camphor when crushed. In spring it produces bright green foliage with masses of small white flowers. It produces clusters of black berry-like fruit around one centimeter in diameter. It has a pale bark that is very rough and fissured vertically.

Camphor is a waxy, white crystalline solid substance, obtained from the tree *Cinnamomum camphora* with a strong aromatic odor. It is terpenoid with the chemical formula $C_{10}H_{16}O$. It is originally obtained by distillation of bark from camphor tree. Camphor has been used for many centuries as a culinary spice, a component of incense, and as a medicine. Camphor is lipophilic in nature which accounts for its rapid movement across mucous membrane and large volume of its distribution. Camphor is synthetically produced from turpentine oil and is present in many non-prescription medicines such as Tiger Balm, Vick's vapor-steam, Bayer Muscle, and joint creams; in addition to many other medicines. Camphor is a natural substance, it was known by Asian nations since ancient times. Its synthetic form is now available and is being produced for medical, sanitary, and industrial usages [7].

Camphor is known for its use for modulating sexual activity, contraception, inducing abortion, and reducing milk production in lactating women. (Goldfrank et al 2002). It has been shown that camphor can easily pass placental barrier and affects development [23]. It is used for its scent, as an ingredient in cooking as an embalming fluid, in religious ceremonies and for medicinal purposes. (Volkel, 2006). Camphor is used not only an aromatic material, but also for different purposes such as stimulation of circulatory and respiratory systems, psychological stimulation, and cosmetics (as sun protection) for external use. [8, 9]. It has been used for centuries as aphrodisiac, contraceptive, abortificient, cold remedy, antiseptic and suppressor of lactation [10]. Also it has been widely used as a fragrance in cosmetics, flavoring food additive, scenting agent in a variety of household products, active ingredient in some old drugs, and intermediate in the synthesis of perfume chemicals [11]. Recently, investigations have shown that camphor containing compounds have uterotrophic [12], antitussive [13], anticonvulsant [14], nicotinic receptor blocking [15], antiimplantaion [16], antiestrogenic [17] as well as estrogenic [17-20] activities, and reduced serum triglyceride and thyroid hormone [21]. In Iran’s folk medicine, camphor has been used both as an aphrodisiac and antiaphrodisiac. In small doses, camphor is used as an aphrodisiac to excite the reproductive organs, causing considerable heat in the urethra and nocturnal emissions. However, in large doses, it is used as an antiaphrodisiac to diminish urino-genital irritation [22]. It has been reported that this process does not have any adverse effects on the woman's ovulation process. Nor does it affect the menstrual cycle but prevent conception. However there are no underlying mechanisms have been reported. The present study is aimed to study the effect of camphor on sperm motility and its viability in *In vitro.* Attempt will also be made to analyze the effects of camphor as one of the local contraceptive method for prevention of conception [23-29].

MATERIALS AND METHOD

To study the *Invitro* affect of different concentration of camphor on human sperm motility, viability and to analyze the effect of camphor and vis-à-vis its use as prospective local contraceptive method for male.

Preparation of camphor solution: - Take 100 gm of camphor crust it in fine powder add to sterile conical flask. Add 100 ml of chloroform to each conical flask. Mix well with the help of stirrer.

Procedure for Sperm motility:-Fresh semen samples were collected from a healthy single donor to avoid inter-donor variability. Check pH of semen sample. Seminal fluid was collected in a clean container and kept warm (body temperature) until used for analysis. The semen was tested for liquid consistency before proceeding with the test. The sample that not liquefied was checked at 10 minute intervals until it

liquefied. The efficacy of 10 µl of sperm suspension and 10 µl of camphor solution (1:1) was tested at different concentrations (1, 2.5, 5 and 10%) and placed on a glass slide and was mixed uniformly. As a control, 10 _l of Ham's-F10 solution was used. Immediately the specimen was examined under the high power objective (40x). The motility of sperm was observed at various time intervals starting from 0 sec and once in every 15 seconds up to 150 seconds. Sperm motility was determined by counting all motile and immotile spermatozoa in several randomly chosen fields using a 40x objective. Only free spermatozoa were assessed. Samples with more than 25% of sperm clumps were not assessed. Spermatozoa with abnormalities such as pin-heads and “free tails” are not counted. Approximately 200 sperms were observed and classified according to their motility (defined as a percentage). The percentage motility per sample was calculated based on the following formula:

$$\text{Motility} = \frac{\text{Motile sperms} \times 100}{\text{Motile + non motile sperms}}$$

Procedure for Sperm Viability: - One drop of Eosin y stain was added in vial. One drop of semen sample with different camphor concentration was added to it at room temperature and was kept undisturbed for one to two minutes and then mixture was smeared on a microscopic slide. 100 spermatozoa were classified as either colored, if the stain has passed through the membrane and therefore the cell was considered dead, or non-stained, the cell than being considered alive. The staining technique helps to differentiate spermatozoa that are immotile but alive from those that are dead. The percentage viability per sample was calculated based on the following formula:

$$\text{Viability} = \frac{\text{Viable sperms} \times 100}{\text{Viable + non viable sperms}}$$

RESULT AND DISCUSSION

The effects of Camphor on the motility of sperm are presented in Table 1 and Figure 1. The result is the proportion of motile spermatozoa, expressed as an integer percentage. It was observed that as concentration of the camphor solution increased, decrease in motility of sperms was observed. The effects of Camphor on the viability of sperm are presented in Table 2 and Figure 2. The result is the proportion of viable spermatozoa, expressed as an integer percentage. It was observed that as concentration of the camphor solution increased, decrease in viability of sperms was observed. Birth control is a regimen of one or more actions, devices, sexual practices, or medications followed in order to deliberately prevent or reduce the likelihood of pregnancy or childbirth. There are three main routes to preventing or ending pregnancy before birth: the prevention of fertilization of the ovum by sperm cells ("contraception"), the prevention of implantation of the blastocyst ("contragestion". Spermatozoa acquire the ability to fertilize an oocyte when they become capacitated. Capacitation takes place when sperm pass through the female reproductive tract, interacting with female fluids. Since capacitation enables the sperm–oocyte interaction, the aim of the present study was to investigate the effect of camphor to disable sperm motility and serve as an effective contraceptive [30].

Contraception (birth control) prevents pregnancy by interfering with the normal process of ovulation, fertilization, and implantation. There are different kinds of birth control that act at different points in the process. The use of different types of contraceptives has various disadvantages depending on the contraceptive. The different contraception methods have many side effects on its use on both male and female. The oral contraceptive has many disadvantages like fluid retention, irregular bleeding, amenorrhea, nausea and vomiting, headache, multiple drug interactions, several contraindications and many more; barrier contraceptive also has many disadvantages like allergic reactions, vaginal dryness or irritation; and other methods also has some disadvantages like irregular bleeding, pelvic pain,

spontaneous expulsion. The easily available substances and the ayurvedic treatment such as sesame oil and rock salt, margosa wood fumigation, seeds of palash with honey and ghee, etc. it should be used rather than using hormones or complex chemicals which have very adverse side effects on the user. Several plant products inhibit male and female fertility and may be developed into contraceptives. Herbal preparations have been tested for spermicidal action in a number of countries. *Aegle marmelos* Corr. (bael) is a popular medicinal plant and cosmopolitan in distribution. All parts of this plant, viz., root, leaf, trunk, fruit and seed, are used for curing one human ailment or another. It has also been reported that aqueous extracts from the leaves of *A. marmelos* reduced the vitality of human sperms [31].The effect of camphor on male reproductive system in rats showed significant structural changes, including vascularization and proliferation of sexual cells, which affect maturation of seminiferous tubules and reproductive function of testes in mice [8]. A decrease in the rat body weight, testis size, sperm number and mobility with all of the experimental doses (10 and 20mg/kg) of camphor caused morphological changes and a toxic effect on sperm and their mobility [32]. The results of this research indicate that there is a direct correlation between the amounts of the dose used with sperm mobility. The findings of the present study clearly indicate that camphor also have an effect on the motility and viability of the human sperms and points to the prospectives of the use of camphor as local contraceptive, which deserves further investigation.

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Table-1: Effect of camphor on Sperm motility

Treatment	Percent motility of spermatozoa at different time intervals (sec)						
	0	15	30	60	90	120	150
Control	79 ± 1.3	78 ± 2.0	78 ± 1.4	79 ± 2.2	79 ± 1.3	77 ± 2.0	77 ± 2.0
1%	79 ± 1.3a	63± 11	55 ± 1.0	48 ± 1.7	40 ± 2.0	34 ± 1.5	19 ± 1.4
2.5%	79 ± 1.3a	59± 1.6a	52 ± 1.0	43 ± 1.4a	34 ± 1.7	27± 1.1	12 ± 1.7
5%	79 ± 1.3b	50 ± 2.0	46 ± 2.0	37 ± 1.7	25 ± 1.2	16± 1.0	6 ± 1.4
10%	77 ± 1.3b	30 ± 1.2	21 ± 1.8a	12 ± 1.8	14 ± 1.8	4 ±1.3a	NIL

Values are mean + SE

[a] $p < 0.05$ by comparison with control.

[b] $p < 0.01$ by comparison with control.

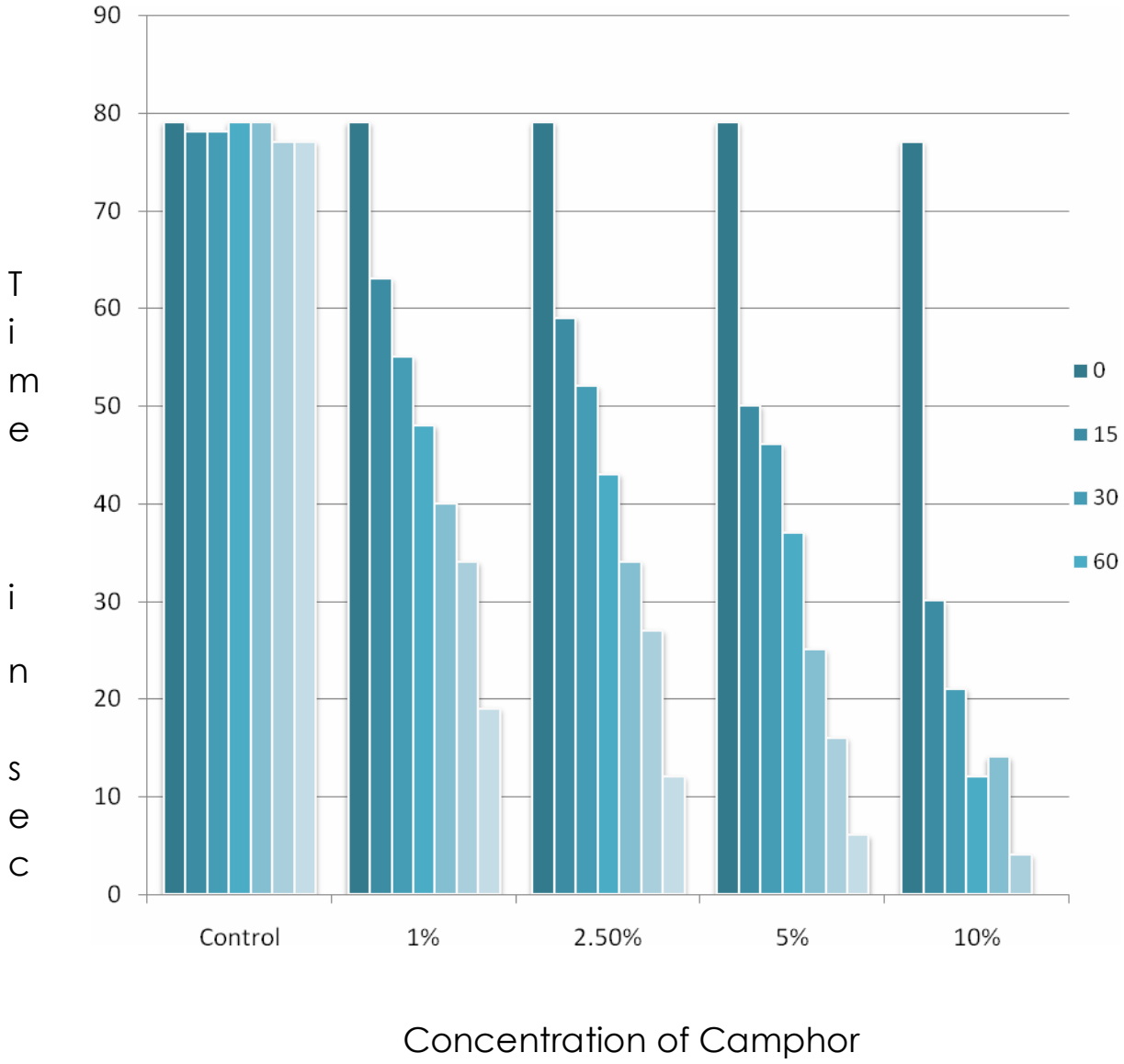

Fig 1:- Graphical representation of Effect of Camphor on Sperm motility

Table 2:- Effect of Camphor on Sperm viability

Treatment	Percent motility of spermatozoa at different time intervals (sec)						
	0	15	30	60	90	120	150
Control	80 ± 1.5	80 ± 2.0	79 ± 1.7	78± 2.2	76 ± 1.8	75 ± 2.4	75 ± 2.4
1%	80 ± 1.5a	72 ± 1.0a	64 ± 1.4a	52 ± 1.9a	44± 2.2a	40 ± 2.0b	17 ± 1.4a
2.5%	80 ± 1.5a	69 ± 1.4a	60 ± 1.3 b	48 ± 1.5a	37 ± 1.7a	29 ± 1.3b	9 ± 1.9a
5%	79 ± 1.7 b	65 ± 1.9b	52± 2.0a	40± 1.7a	28 ± 1.4a	17 ± 1.4a	4± 1.9a
10%	77 ± 1.7 b	50± 1.3	29 ± 1.9	19 ± 1.9	10± 1.5a.	5 ±1.6	NIL

Values are mean + SE

[a] $p < 0.05$ by comparison with control.

[b] $p < 0.01$ by comparison with control.

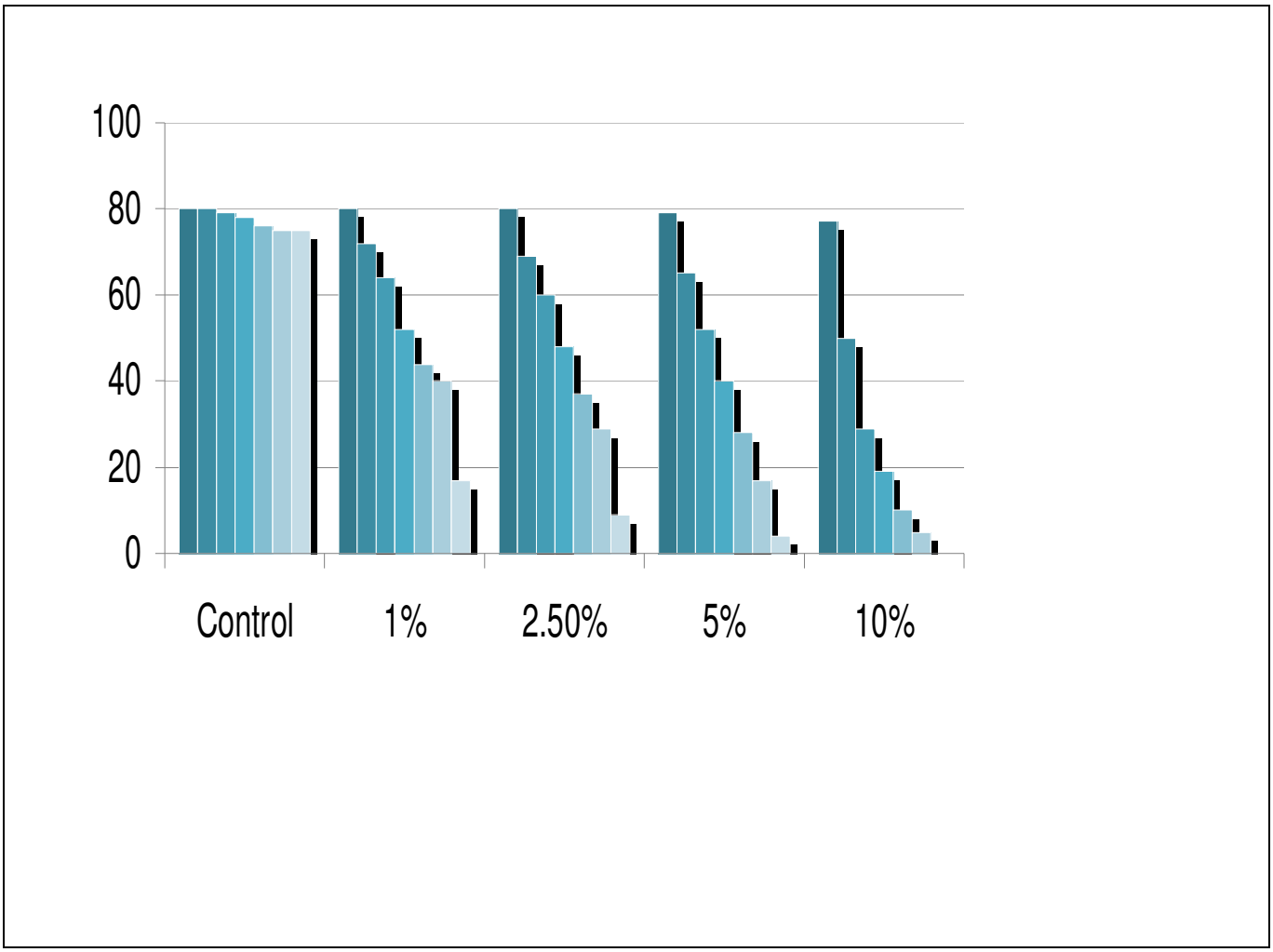

Figure 2:- Graphical representation of Effect of Camphor on Sperm Viablity