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Spermicidal Activity of Neem Oil Fractions

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Neem (Azadirachta indica A. Juss.) is a traditional medicinal plant in India. Neem oil, extracted from the seeds of the neem tree, has been found to possess strong spermicidal activity against rhesus monkey and human spermatozoa, in vitro. On vaginal application, the oil also possesses anti-implantation and abortifacient activities (Sinha et al., 1984). Oral administration of aqueous extract of neem leaf also shows antifertility effect in mice (Deshpande et al., 1980). This study aims to evaluate the in vitro spermicidal activity of neem oil fractions obtained by solvent extraction and column chromatography, against bovine spermatozoa.

Materials and Methods

Neem oil was fractionated by two different approaches viz., extraction by using individual polar and non-polar solvents and by adsorption chromatography using a column of silica gel as per methodology of Barman (2003).

In the first approach, neem oil was subjected to fractionation by extraction with three different solvents from polar to non-polar viz., methanol, butanol and hexane, since neem oil was not completely miscible with these solvents, unlike extremely non-polar solvents like benzene and chloroform. In the second procedure, 10 ml of neem oil was subjected to
fractionation by adsorption chromatography in a column of silica gel (40x2 cm) using a solvent mixture of chloroform : methanol : water (65:35:3). The solvents from the fractions were evaporated to dryness by subjecting the tubes in a vacuum chamber maintained at 50°C. The fractions obtained were then subjected to thin layer chromatography (TLC) to check the number of different lipids present in each fraction. TLC was conducted for whole neem oil as well as different fractions obtained by column chromatography.

Two fractions could be obtained each from methanol and hexane extraction and they were named as F1 (methanol-miscible fraction), F2 (methanol-immiscible fraction), F3 (hexane-miscible fraction) and F4 (hexane-immiscible fraction). However, when 10 ml of neem oil was vigorously shaken with 10 ml of butanol, no separation occurred between the oil layer and the butanol layer, as the mixture became an emulsion; no fraction could therefore be extracted with butanol. The chemical nature of these isolated fractions could not be ascertained in this study, but they appeared to be different substances, since the Rf (Retardation factor) values were different from each other in the same solvent system used. By column chromatography, in total, two fractions were recovered, which were named as F5 (column chromatography fraction 1) and F6 (column chromatography fraction 2), following TLC analysis.

The spermicidal activity of six neem oil fractions (F1, F2, F3, F4, F5 and F6) was determined (Waller et al., 1980). A drop of the liquid semen containing approximately 1020 x 10^6 sperm per ml obtained from healthy bulls (by AV method) was placed on a clean slide. A drop of neem oil fraction was placed close to the drop of semen. The two drops were covered by a cover slip so that a 'bridge' (semen-neem oil interface) was formed, incubated for 20 min and thereafter examined under a microscope. Motility and live and dead counts of the spermatozoa were checked at different time intervals viz., 0 min., 5 min., 10 min, 15 min and 20 min. A similar procedure was followed to study the spermicidal activity with all the neem oil fractions. Sperm motility and live and dead counts were also determined in semen-normal saline interface, which served as controls. The data obtained from the study were subjected for statistical significance by one-way analysis of variance (Snedecor and Cochran, 1994).

**Results and Discussion**

Once the sperms touched the margin of oil fractions, it was found that they get completely entangled, leading to further sluggishness. When tested with normal saline (in the control group), through the sperms became sluggish in motility, complete immotility was attained after more than an hour. It was further observed that bovine semen when treated with F1, 30% spermatozoa remained motile even after 20 min of incubation. On the contrary, in the other five fractions, motility percentage after 20 min reduced upto 5% (F2) or below 5% (F3, F4 and F6). No motile sperm could be observed in semen sample treated with F5 after 20 min of incubation. The spermatozoa motility with F1 was thus, significantly higher (p<0.05) than the other five fractions.

When bovine semen was treated with F1, 34% sperms remained live even after 20 min of incubation, as compared to the other fractions, where livability percentage reduced to below 10%. Sharma and Saksena (1959) in their in vitro study on human spermatozoa reported a weak spermicidal action of sodium nimbidinate, a derivative of neem oil (spermatozoa killed within 30 min). Sinha et al. (loc. cit) reported that spermatozoa of human and monkey became immotile within 30 sec. of contact with neem oil. Riar et al. (1990), reported that a volatile fraction of neem oil (NIM – 76), obtained by steam distillation, even when used at its lowest concentration, i.e., 0.25 mg/ml, the spermatozoa were rendered immotile within a period of 20 sec. of mixing, indicating a strong spermicidal activity.
In the present study, F5 appeared to be the most potent spermicidal fraction, while the fraction having least spermicidal activity (p<0.05) was F1. However, all the fractions tested appeared to have potent spermicidal activities.

**Summary**

The present study was undertaken with a view to assess in vitro spermicidal property of neem oil fractions. When semen was treated fraction with F1, 30% spermatozoa remained motile (p<0.05) even after 20 min unlike the other five fractions in which motility percentage after 20 min reduced upto 5% (F2) or below 5% (F3, F4 and F6). No motile sperm could be observed with F5 after 20 min of incubation. Likewise, the livability percentage of spermatozoa with F1 after 20 min was found to be significantly higher than the other fractions in which livability percentage reduced to below 10%. It is concluded from the present study that the spermicidal activity of the methanolic fraction of neem oil (F1) is significantly lower than the other fractions.

**References**


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**Prediction of Live Body Weight in Mahabubnagar Goats***


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Mahabubnagar goats, which are distributed widely in southern Telangana region of Andhra Pradesh, are well known for their meat production potential and meat quality. Information on the prediction of body weights of Mahabubnagar local goats is scanty. Since there is a relationship between the body weights and body measurements the present study was undertaken to develop the multiple regression equations using different body measurements to predict the live body weight in Mahabubnagar goats.

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