EVALUATION OF EFFICACY, INTRAUTERINE SAFETY AND STORAGE CONDITIONS OF LUGOL'S IODINE

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ABSTRACT

Different concentrations of Lugol's iodine [I_2KI] have been utilized to treat endometritis with variable success rates in cows. In present study, intrauterine infusion of 30 ml of 0.3% I_2KI during estrus and one day later revealed a therapeutic success rate of 56.2% in 16 cycling cows with moderate moderate/severe clinical endometritis. The cows not responding tended to have a greater magnitude of uterine microbial load before treatment. The same protocol investigated in another set of six cows with healthy uterus revealed a normal endometrial histology in the estrus succeeding I_2KI infusion, thereby suggesting the said protocol to be safe.. In another part of study, I_2KI stored in transparent or dark glass containers for a period of 6 months and exposed to illumination intensity of 69.5 ± 2.3 lux did not affect the *in vitro* antibacterial activity. However, an air exposure for 72 h significantly reduced the optical density and *in vitro* antibacterial activity.

Key words: Cattle, Endometritis, Lugol's iodine, Storage.

Endometritis is the most common cause of conception failure involving high percentage of bovines in India (Shukla and Pandit, 1989). Antibiotics are quite effective in treating endometritis, but *in vitro* sensitivity, accurate dosage regimens (Sood, 1995) and compulsory milk disposal (Hussain and Daniel, 1991) make I_2KI the most viable preposition. Low I_2KI concentrations of 0.1 and 0.2%, mostly being used, are hypothesized to suffer from reduced clinical cures, which call for testing of higher I_2KI concentrations. The topical iodine solutions in an alcoholic base have a definite shelf life that depends on the storage conditions (Reynolds, 1989). There is no such information for the aqueous based iodine solutions such as, I_2KI. Hence, the present study intended – (1) to evaluate higher I_2KI concentration for therapeutic success and post-treatment endometrial regeneration and (2) to study the effect of certain storage conditions of I_2KI on its *in vitro* antibacterial efficacy and/or optical density.

The work was done in cycling cows with moderate / severe clinical endometritis (Group I; n =16) and others with a healthy uterus (Group II, n = 6). In both groups, 30 ml of 0.3% I_2KI was infused in uterus during estrus and 24 h later. The genital discharge was aspirated aseptically from uterus before and after treatment (in subsequent estrus) in Group I to diagnose the endometritis severity, clinical recoveries (Roberts, 1986) and to evaluate a change in colony forming units (CFU)/ ml performed by Standard Plate Count Method (APHA, 2001). In Group II, endometrial biopsy was collected from the ventral surface of the uterine horn with a sterile Neilson's Uterine Biopsy Punch before I_2KI infusion and in the subsequent estrus for assessment of endometrial regeneration by using routine Haematoxylin and Eosin technique (Luna, 1968).

To study the effect of illumination on storage, two sets of fresh I_2KI stock solution (5%), prepared on the

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same day, were stored separately in 500 ml transparent and illumination resistant containers, respectively, for six months. Intensity of illumination (in lux) of the storage area was measured thrice as week with the help of a Lux Meter (National Industrial Corporation, Kolkata, India). After six months, the I_2KI stored in the two different bottles were diluted to 0.2, 0.3, 0.4 and 0.5%, respectively, each one of which was evaluated for *in vitro* antibacterial activity against similar, but freshly prepared I_2KI concentrations. To evaluate the effect of air exposure on I_2KI, 0.2 to 0.5% I_2KI concentrations were prepared and stored separately in 20 ml glass vials (three each for different concentrations) with either tight or half - loose screw caps for a period of 72h. The optical density (OD; measured in a spectrophotometer at a wavelength of 550 nm) and antimicrobial activity of I_2KI were compared between corresponding I_2KI concentrations of freshly prepared and I_2KI stored in tight as well as loose screw cap vials at three different intervals of 0 h (immediately after reconstitution), 48h and 72h after reconstitution, respectively.

The *in vitro* antibacterial activity of I_2KI was determined against a pure culture of *Staphylococcus aureus* (ATCC 6538) using standard Cylinder – Plate Bioassay Technique (Arret *et al.*, 1971). The antibacterial activity was assessed in terms of size of zone of inhibition (ZOI; in cm), greater the ZOI higher the antibacterial activity.

The statistical analysis comprised of Student's *t*-test (Snedecor and Cochran, 1989).

In Group I, 9 out of 16 cows (56.2%) recovered from endometritis, which equals the success of 54.1% following single uterine infusion of 30 ml of 0.1% I_2KI in cows (Sood *et al.*, 2000). Higher success of 65.4% using 30ml of 0.25% I_2KI in cows with sub-clinical endometritis (Shukla and Pandit, 1989) could be incriminated to higher I_2KI concentration and low grade endometritis in the latter study. In Group I, the average CFU/ml tended to reduce in cows responding to treatment ($9.3 \times 10^4 \pm 5.6 \times 10^4$ vs. $4.4 \times 10^2 \pm 1.0 \pm 10^2$; P = 0.10), whereas there was not much change in the CFU status in the others failing to respond ($3.8 \times 10^6 \pm 3.2 \times 10^6$ vs. $8.2 \times 10^5 \pm 6.8 \times 10^5$). This implies a low bacterial load to dictate clinical cures. However, variation in the pathogen, infection from a single or multiple microbes (Tizard, 1996) cannot be precluded.

In all cows of group II, the endometrial biopsies before and after I_2KI infusion were histologically similar (epithelium, vasculature and endometrial glands) and simulated to the findings during estrus in cows (Dellman and Brown, 1987). Hence, 0.3% I_2KI is clinically safe.

The intensity of illumination during the course of investigation was 69.5 ± 2.3 lux (range of 61 to 80 lux), which did not affect *in vitro* antibacterial activity of I_2KI. The average ZOI (cm) for fresh vs. I_2KI stored in illumination resistant or transparent containers was similar and ranged from 0.05 ± 0.01 to0.07 ± 0.01, 0.17 ± 0.01 to 0.25 ± 0.01, 0.32 ± 0.01 to 0.33 ± 0.01 and 0.42 ± 0.02 to 0.45 ± 0.01 for 0.2, 0.3, 0.4 and 0.5% I_2KI, respectively. Our observation of no effect of illumination is contrary to Reynolds (1989) who recommended storage of iodine containing solutions in illumination resistant containers. Hence, it may be inferred that illumination of intensity greater than that in present study may affect the clinical efficacy of I_2KI.

All concentrations of I_2KI stored with a loose lid revealed a reduced - colour intensity (as visualized by naked eye), O.D and *in vitro* antibacterial activity over a 72h period. However, the 0h, 48h and 72h samples of different I_2KI concentrations prepared fresh, stored in air tight containers and 0h samples stored with loose lid were similar for O.D and antibacterial activity. Hence, the O.D and ZOI of the 48h and 72h I_2KI samples stored with a loose lid were compared with the 0h values of same group. For 0.2% I_2KI stored with loose lid, the O.D at 0h significantly ($P<0.01$) reduced by 48h and further by 72h (0.74 ± 0.001 vs. 0.68 ± 0.001 and 0.51 ± 0.001). Similar significant differences were recorded for 0.3% (1.01 ± 0.001 vs. 0.85 ± 0.001 and 0.69 ± 0.001), 0.4% (1.32 ± 0.001 vs. 1.10 ± 0.001 and 0.88 ± 0.001) and 0.5% (1.61 ± 0.001 vs. 1.35 ± 0.001 and 1.19 ± 0.001) I_2KI concentrations. A decrease in O.D was paralleled by reduced ZOI. For 0.2% I_2KI stored with loose lid, the ZOI (cm) at 0h was significantly ($P<0.01$)

reduced by 48h and 72h (0.07 ± 0.01 vs. 0.04 ± 0.001 and 0.001 ± 0.0001). Similar significant differences were recorded for 0.3% (0.16 ± 0.01 vs. 0.10 ± 0.001 and 0.05 ± 0.001), 0.4% (0.32 ± 0.01 vs. 0.28 ± 0.001 and 0.19 ± 0.001) and 0.5% (0.44 ± 0.01vs. 0.40 ± 0.001and 0.20 ± 0.001) I_2KI concentrations. The change in antibacterial activity on exposure to air is attributed to (i) conversion of I_2KI to IO_3 (iodate) / IO_4 (iodic acid) having reduced antibacterial activity than I_2KI and (ii) vaporization of iodine (Baker *et al.*, 2001).

In conclusion, 0.3% I_2KI is safe for clinical use. Increased quantum of uterine infection may reduce the response to I_2KI. Furthermore, I_2KI can be stored in transpired glass bottles under room conditions without a reduction in antibacterial activity for a period of six months. However, I_2KI has to be stored in air tight containers with minimum of air space.

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