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Evalution of therapeutic efficacy of *Nigella sativa* and *Azadaricta indica* in subclinical mastitis in buffaloes

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Abstract
For the study a total of 24 buffaloes suffering for subclinical mastitis were randomly divided into four groups comprising of 6 animals in each group, besides this, six animals were kept as healthy control (Group-A). Under the experiment *Azadirachta indica* (Neem) sterilised methonolic seed extract @ 700mg/5ml phosphate buffer saline for 5 days and *Nigella sativa* (kalonji) sterilised methonolic seed extract @ 10ml 6% solution in liquid paraffin for 5 days were used intramammary as well as topically in different groups. Amongst the various groups under therapy, the results revealed that group third in which *Nigella sativa* (intramammary and topically) was given found to be the best in respect of maximum number of animals recovered (5/6) on 7th, 15th and 30th days post treatment with significantly decreased milk pH (6.85 ± 0.23*) and decreased Somatic Cell Count (3.21 ± 0.76*x 10^5 cells/ml) with better clinical recovery.

Keywords: *Nigella sativa*, *Azadirachta indica*, subclinical mastitis, buffaloes

Introduction
Ethnoveterinary medicine was defined by McCorkle in 1995 as: The holistic, interdisciplinary study of local knowledge and its associated skills, practices, beliefs, practitioners, and social structures pertaining to the healthcare and healthful husbandry of food, work, and other income-producing animals, always with an eye to practical development applications within livestock production and livelihood systems, and with the ultimate goal of increasing human well-being via increased benefits from stock raising.
In Madhya Pradesh, livestock farmers which are poor and lives in remote areas generally use ethnoveterinary practices or medicinal plants for the treatment of their animals. Among various medicinal plants, is emerging as a miracle herb with a rich historical and religious background since many researches revealed their wide spectrum of pharmacological potential. The present study was therefore performed to evaluate the efficacy of *Nigella sativa* (*Black seed*) and *Azadaricta indica* (Neem) in the treatment of subclinical mastitis in buffaloes.

Materials and Methods
Twenty four buffaloes with subclinical mastitis were randomly selected and divided into four groups with six animals in each and treated as per the schedule (Table 1).

Table 1: Group wise therapeutic schedule in subclinical mastitic buffaloes

Group	No. of animals	Drugs
A	6	Healthy control
B	6	*Azadaricta indica* SMSE @ 700 mg/5 ml PBS IMM q12 h for 5 days + *Nigella sativa* SMSE topically per 12 h for 5 days
C	6	*Nigella sativa* SMSE @ 10 ml 6% solution in liquid in paraffin IMM per 12 h for 5 days + *Azadaricta indica* SMSE topically q12 h for 5 days
D	6	*Nigella Sativa* SMSE @ 10 ml 6% solution in liquid in paraffin + IMM and topically per 12 hr interval for 5 days
E	6	*Azadaricta indica* SMSE @ 700 mg /5 ml PBS. IMM and topically per 12 hr interval for 5 days

*SMSE- Sterilized methanolic seed extract; IMM- Intramammary

For preparation of methanolic extract of *Azadirachta indica*, seeds were washed, dried and grounded to a coarse powder. The seed powder was loaded in to Sauxlet apparatus and extracted with 70% methanol (yield 14.28% w/v) dried under vacuum below 40°C. The condensed herb was reconstituted in sterile PBS, (10 mm, pH 7.4) having 700 mg extract /5 ml PBS as per the standard method (De and Mukherjee, 2009) [1]. For *Nigella sativa* methanolic extract, 100 gm of black seed powder was used with 600 ml of methanol for an extraction of 12 hours. The extract was filtered with Whatmann filter paper and the solvent was evaporated by rotary distillation apparatus. In order to obtain a complete dry extract, the resultant extract was transferred to glass dishes and left at 50 °C for 24 hours in hot air oven as per the standard procedure (Monika *et al*., 2013) [2].

Confirmation of presence of subclinical mastitis was done by using California mastitis test on day 0 pretreatment and day 30 post treatment. Therapeutic efficacy was judged by milk pH, and somatic cell count on days '0' before treatment and days 7, 15 and 30 after treatment. Data collected from the study were subjected to "ANOVA and Duncan's multiple range test" as per standard statistical method (Snedecor and Cochran, 1994) [3].

Results and Discussion

Milk pH values

The mean milk pH under control group "A" remained almost normal however, in group "B", "C", "D", "E" the pre treatment (day 0) pH values decreased significantly on 7th, 15th and 30th day post treatment (Table 2).

Table 2: Changes in pH in milk after treatment of Subclinical mastitis (Mean ± SE)

S. No.	Group	Pre treatment	Post treatment		
		0 day	7th day	15th day	30th day
1	A (c)	6.53 ± 0.08^{P}	6.43 ± 0.08^{P}	6.43 ± 0.07^{P}	6.32 ± 0.07^{P}
2	B	7.73 ± 0.05^{AQR}	7.45 ± 0.05^{BQ}	7.18 ± 0.10^{BQ}	7.00 ± 0.12^{CQ}
3	C	7.68 ± 0.06^{AQR}	7.50 ± 0.06^{BQ}	6.98 ± 0.12^{BQ}	6.72 ± 0.14^{AQR}
4	D	7.60 ± 0.09^{AR}	6.68 ± 0.17^{BP}	6.57 ± 0.15^{BP}	6.48 ± 0.17^{BPR}
5	E	7.80 ± 0.05^{AQ}	7.60 ± 0.05^{BCQ}	$7.32 \ 0.11^{BQ}$	7.07 ± 0.17^{ACQ}

*(c) – Control; Value with different superscript within the group (PQR: $p<0.01$) and between the group (ABCD: $p<0.01$) differ significantly

Somatic cell count values

The mean milk SCC under control group "A" remained almost normal, however, in group "B", "C", "D", "E" the pre treatment (day 0) SCC values were 7.69 ± 0.63, 8.22 ± 0.15, 7.79 ± 0.45 and 7.23 ± 0.41 respectively, which decreased significantly on 7th, 15th and 30th day (Table 3).

Table 3: Changes in SCC, in milk after treatment of Subclinical mastitis (Mean ± SE)

S. No.	Group	Pre treatment	Post treatment		
		0 day	7th day	15th day	30th day
1	A (c)	1.09 ± 0.08^{P}	1.32 ± 0.08^{P}	1.12 ± 0.06^{P}	1.09 ± 0.08^{P}
2	B	7.69 ± 0.63^{AQ}	6.00 ± 0.9^{BQ}	4.77 ± 0.88^{CQ}	2.95 ± 0.18^{DQ}
3	C	8.22 ± 0.15^{AQ}	5.93 ± 0.72^{BQ}	3.81 ± 0.68^{BQ}	2.68 ± 0.11^{CQ}
4	D	7.79 ± 0.45^{AQ}	3.21 ± 0.76^{BR}	2.45 ± 0.33^{BR}	1.55 ± 0.29^{BR}
5	E	7.23 ± 0.41^{Q}	6.23 ± 0.53^{Q}	4.94 ± 0.41^{Q}	3.18 ± 0.29^{Q}

*(c) – Control; Values with different superscript within the group (PQR: $p<0.01$) and between the group (ABCD: $p<0.01$) differ significantly

Therapeutic efficacy of herbal drugs on the basis of California mastitis test

The overall results of the therapeutic study indicated that the therapeutic efficacy of the drug used under group "D" (*N. sativa*) was found to be maximum efficacious (83.33%). However, the drugs used in group "C", "B" and "E" with different routes were reported to be 66.66%, 50% and 33.33% effective respectively (table 4).

Table 4: Comparative Efficacy of therapeutic agents on the basis of California mastitis test

Name of drug, and administration	Group	No. of Animals treated	No. of animals cured	Percentage recovery
(SMSE) *A. indica* (IMM) + (SMSE) *N. sativa* (topically)	B	6	3	50
(SMSE) N. sativa (IMM) + (SMSE) *A. indica* (topically)	C	6	4	66.66
(SMSE) *N. sativa* (IMM & topically)	D	6	5	83.33
(SMSE) *A. indica* (IMM & topically)	E	6	2	33.33

During the present investigation, the mean milk pH in buffaloes affected with subclinical mastitis was significantly higher (7.28 ±0.11) as compared to apparently healthy control group. Other scientist also showed that the milk pH from severely affected buffaloes was 7.18 (Kumar, 2011) [4]. This might be due to the leakage of blood bicarbonate into milk through damaged epithelium of mammary gland (Bansal and Randhawa, 2003) [5].

The mean milk SCC values of apparently healthy buffaloes were 1.09 ± 0.08 x 10^5 cells/ml. There was a significant increase (8.22 ± 0.15 x 10^5 cells/ml) in the mean values of SCC in the animals affected with subclinical mastitis. These observations were in agreement with other reports who found the SCC value in subclinical mastitic milk as 11.48±0.73 x 10^5 cells/ml, because the inflammation of udder might lead to high SCC in milk (Seriys, 1985) [6].

The overall findings of the present investigation indicated that the buffaloes treated with injection of 10 ml 6% paraffin solution of the *Nigellia sativa* methanolic seed extract twice daily 5 days by intramammary route along with its topical application, induced an excellent response by producing stronger effects against the teat skin opportunistic organisms

(Chavoshi and Husaini, 2012) [7], leading to clinical recovery in maximum buffaloes under the group "D" in comparison to *Azadirichta indica* as a therapy for subclinical mastitis in buffaloes. The results are suggestive of strong antimicrobial and anti-inflammatory effect of *Nigellia sativa* on microorganisms.

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