Collection, documentation and validation of some ethnic biodynamic agents used by the tribal communities of West Singhbhum District of Jharkhand against bovine mastitis

Thesis

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy
(Veterinary Medicine)

August, 2014

Dedicated to....

My beloved Parents, Brother

&

Husband

भारतीय पशु चिकित्सा अनुसंधान संस्थान
(सम विश्वविद्यालय)
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Dated: 28.8.2014

Certificate

This is to be certified that the research work embodied in this thesis entitled **"Collection, documentation and validation of some ethnic biodynamic agents used by the tribal communities of West Singhbhum District of Jharkhand against bovine mastitis"** *submitted by* **Dr. Rajni Prabha Mahto, Roll No. 1472,** *for the award of* **Doctor of Philosophy** *Degree in* **Veterinary Medicine** *at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate herself under my supervision and guidance.*

It is further certified that **Dr. Rajni Prabha Mahto, Roll No. 1472,** *has worked for more than 30 months in the Institute and has put in more than 300 days attendance under me from the date of registration for the* **Doctor of Philosophy** *Degree in this Deemed University, as required under the relevant ordinance.*

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Certificate

We the undersigned members of Advisory Committee of **Dr. Rajni Prabha Mahto, Roll No. 1472,** *a candidate for the degree of* **Doctor of Philosophy** *with the major discipline* **Veterinary Medicine,** *agree that the thesis entitled* **"Collection, documentation and validation of some ethnic biodynamic agents used by the tribal communities of West Singhbhum District of Jharkhand against bovine mastitis"** *may be submitted in partial fulfillment of the requirement for the degree.*

We have gone through the contents of the thesis and are fully satisfied with the work carried out by the candidate, which is being presented for the award of **Doctor of Philosophy** *Degree of this Institute.*

It is further certified that the candidate has completed all the prescribed requirements governing the award of **Doctor of Philosophy** *Degree of the Deemed University, Indian Veterinary Research Institute, Izatnagar.*

28.11.14

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Date: 28.8.2014

Place: Bareilly

Rajni Prabha Ma

(Rajni Prabha Mahto)

ABBREVIATIONS

%	:	Percentage
µl	:	Microlitre
ABST	:	Antibiotic Sensitivity Test
ANOVA	:	Analysis of variance
BW	:	Body Weight
cfu	:	Colony Forming Unit
CM	:	Clinical Mastitis
CMT	:	California Mastitis Test
CNS	:	Coagulase Negative *Staphylococcus aureus*
CPS	:	Coagulase Positive *Staphylococcus aureus*
DLC	:	Differential Leukocyte Count
DPX	:	Di-n-butyl phthalate in Xylene (a mountant used in histopathology)
DW	:	Distilled Water
E.coli	:	*Escherichia coli*
EVM	:	Ethno Veterinary Medicine
EVP	:	Ethno Veterinary Practices
I/V	:	Intravenous
IMI	:	Intramammary infection
ITK	:	Indigenous Traditional Knowledge
IU	:	International Unit
L-4/L_4	:	Left fourth
LAR	:	Laboratory Animal Resource
LPM	:	Livestock Production and Management
MIC	:	Minimum Inhibitory Concentration
mm	:	Millimeter
NMC	:	National Mastitis Council
NSS	:	Normal Saline Solution
PBS	:	Phosphate Buffer Saline
PC	:	Post Challenge
PCV	:	Packed Cell Volume
PMNs	:	Polymorphonuclear Neutrophils
PRA	:	Participatory Rural Appraisal

QuIK	:	Quantification of Indigenous Knowledge
S. aureus	:	*Staphylococcus aureus*
SCC	:	Somatic Cell Count
SCM	:	Subclinical Mastitis
SD	:	Standard Deviation
St. agalactiae	:	*Streptococcus agalactiae*
TCS	:	Total Clinical Scores
v/v	:	volume / volume
WHO	:	World Health Organization
WTO	:	World Trade Organization

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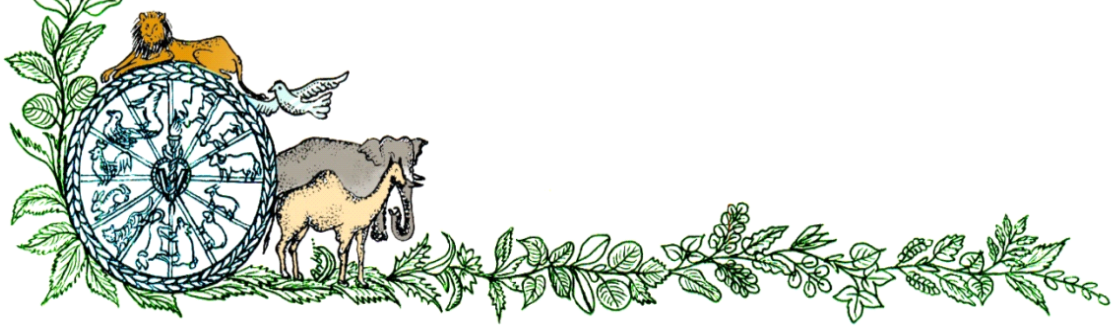
Introduction



1 INTRODUCTION

1.1 Ethnoveterinary medicine (EVM)

Ethnoveterinary medicine (EVM) is a traditional system of medicine which is locally available and cheap, dealing with local belief, skill, knowledge, methods and practices related to healthcare of animals (McCorkle, 1986; McCorkle *et al*., 1996). Ancient man had discovered natural products to satisfy his needs including relief from his personal ailments as well as his domestic animals. Ethnoveterinary veterinary medicine is the holistic interdisciplinary study of the local knowledge and the socio-cultural structures and environment associated with animal healthcare and husbandry. Information regarding art of caring for animals was provided by the sacred texts of the Vedic religion. The oldest Vedic literature consists of collections of hymns, liturgical chants and sacrificial or magical formulae, mainly in verses, which constitute the Veda proper 1,500-1,000 BC (Mazars, 1998). The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments (Rabe and Staden, 1997). Since time immemorial, animal and plant relationship has been continuing. Local people prefer these time tested herbal medicines to treat their ailing cattle, Tribal human societies developed the system of therapy from the keen observation of the ailing animals and their dietary behaviour. Thus the knowledge over which plants were beneficial for which ailments grew and the persons who gathered this information were became the specialists. Later they might have experimented with these plant/plant parts for such ailments (Fielding, 1998). Nakula Samhita is considered the first treatise dealing with treatment of animals with herbal preparations during the Mahabharata period. Inscriptions of Ashoka's period

indicate the existence of veterinary hospitals which were supported by the king and also the existence of specialists for treatment of various categories of animals like horses, elephants, cows and birds (Majumdar, 1989). Traditional animal doctors are a substantial component of livestock healthcare systems in developing countries. Such healers and their roles have been largely ignored by the modern veterinary community. Little that is known about traditional livestock healers and their practices is that they represent a valuable, but as yet untapped, resource for extending many aspects of basic animal healthcare, especially to poor and smallholder producers in remote or difficult environments (Anonymous, 1997).

Traditional herbal healers who acquired indigenous knowledge of the veterinary health care system are transferred by word of mouth from one generation to other (Phondani *et al*., 2010). The traditional knowledge on ethnoveterinary practices by Vaidya and local healers, who are knowledgeable and experienced in traditional systems of treatment, but their knowledge is not documented, and is dwindling fast (Jain and Srivastava, 1999). Indigenous Traditional Knowledge (ITK) is an integral part of the culture and history of a local community. It is evolved through many years of regular experimentation on the day to day life and available resources surrounding by the community. It is the unique, traditional, local knowledge existing within and developed around specific condition of men and women indigenous to a particular geographical area.

Traditional veterinary medicine is very important in developing countries where conventional remedies for animal health care are inaccessible or unaffordable to poor rural farmers. High costs and inaccessibility have helped to maintain traditional treatment practices in these countries and fostered research on this subject (Muhammad *et al*., 2005). In developing countries much effort is needed in research and integration of the ethnoveterinary practices (Mathias and McCorkle, 1997).Where ethnoveterinary practices can lead for their validation and eventually to better animal healthcare provision and enhanced living standards of the rural folks (Nyamanga *et al*., 2008; Lans *et al*., 2007).

Forests are associated with socio-economic and cultural life of tribal's in India. These tribal areas inhabit wide ecological and geo-climatic conditions and are scattered

throughout the country. Tribal livelihood system varies considerably amongst different regions among the various ethnic groups, depending on ecological, historical and cultural factors. The word 'Jharkhand' means the 'land of forests' or the 'forested area'. The area covered with forest stretched from the north of Jharkhand and through its centre to the south and southwest, which is the West Singhbhum district. Tribals constitute around 28 % of total population of the state of Jharkhand, which is around 8% of total tribal population of India. Among the 32 tribes in Jharkhand, nine tribes have been classified as Primitive Tribe Groups (PTG). They are: Asur, Birhor, Birajia, Korwa, Parahiya (Baiga), Sabar, Mal Pahariya and Souriya Pahariya and Bil Kahria. In Jharkhand, the PTG population is just 2.23 lakh as per 2011 Census. They are primarily hunters and gatherers. The livelihood options of these primitive tribal groups are broadly classified as agriculture, forestry, handicrafts and handlooms. Since tribal communities live in close proximity with biodiversity rich landscapes, they have evolved local specific and novel livelihood strategies based on their ITKs. This knowledge is passed on through generations and it plays an important role in the conservation and sustainable use of biodiversity. Social and cultural diversity, coupled with the environmental complexity, have generated diverse approaches and technologies in the management and use of different natural resources in Jharkhand (Mishra, 2007). The harmful effects of the modern medicine and its ineffectiveness against many chronic diseases have resulted in resurgence of herbal medicine. However, despite its rich herbal wealth, India's share in the world market is even less than 1.5%. The basic requirements for gaining entry into developed countries herbal trade have been assessed as: well documented knowledge about therapeutic efficacy of Indian crude drugs, absence of harmful contaminants, viz. residues of pesticides, heavy metals, micro-organisms etc., quality formulations which have been standardised in respect of not only their physical and chemical characters and therapeutic efficacy but for their safety also. Herbal remedies used for hundreds of years by stock raisers can be put to commercial use, but traditional knowledge should be validated, to verify the safety and efficacy of the treatments.

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence,

often for hundreds of years, before the development and spread of modern medicine and are still in use today (Gansser, 1964). Herbal medicines are very cheap in comparison to the conventional form of medication. One of the greatest benefits associated with herbal medicine is the lesser side effects. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. Jharkhand has a forest area of 23605 square kilometers, which is 29% of its total geographical area. West Singhbhum district of Jharkhand is a remotely located area, rich in biodiversity of medicinal plants and their traditional usage (Mairh *et al*., 2010). The total population, unique topography, climatic conditions and large forest cover makes the locale very rich in medicinal and aromatic plants diversity as an attraction for the explorers. Native ethnic groups, difficult topography and a long time span encompassing hundreds of generations set the perfect stage for the highly evolved form of traditional knowledge to take shape and makes Jharkhand an ethnobotanist's delight.

1.2 Bovine Mastitis

Mastitis, an inflammation of the mammary gland for infection of contagious and environmental bacteria is one of the most important diseases in dairy animals (Viridis *et al*., 2010; Zhu *et al*., 2007). Infection of the cow's udder (bovine mastitis) has remained one of the major constraints in growth of dairy industry in India and abroad. Amongst cattle diseases, bovine mastitis is a serious problem which affects the basic income of the farmers depleting their dairy sources (Mubarack *et al*., 2011; Al Qumber and Tagg, 2006). It is also one of the most costly diseases confronting the dairy farmer. Estimating economic losses resulting from mastitis becomes an extremely difficult task because of the many levels of infection and other factors. Huge financial losses occur due to mastitis, it is a global problem and adversely affects animal health, and quality of milk and economics of milk production and every country including developed ones suffer (Sharma *et al*., 2007). Heavy economic losses occur due to mastitis, it is most important deadly disease of dairy animals and is responsible for reduced milk yield (up to 70%), milk discard after

treatment (9%), cost of veterinary services (7%) and premature culling (14%) (Bhikane and Kawitkar, 2000).

The continuous use of antibiotics for a long period may lead to multi drug resistance in causative organisms which poses the danger of increasing amount of antibiotic residues in milk, a potential hazard (Gopinath *et al*., 2011). Medicinal plants have been used for ages in developing countries as alternative treatment to health problems. India has a diverse flora and a rich tradition in the use of medicinal plants for antimicrobial applications. Many plant extracts have been shown to exert biological activity in vitro and in vivo, justifying research on traditional medicine focused on the characterization of antimicrobial activity of these plants (Mubarack *et al*., 2011).

1.3 Mouse model mastitis

Mouse models are better options to study the mechanisms of pathogenesis, immune responses and the control of mastitis induced by different pathogens *viz. Staphylococcus aureus* and *Escherichia coli* (Brouillette and Malouin, 2005). There is record of only few murine studies on *Streptococcus agalactiae* as mastitis causing organisms (Notebaert and Meyer, 2006). Differences may occur between ruminant and murine mammary glands (Notebaert and Meyer, 2006), both cattle and mice have glands which are functionally and anatomically independent from each other. Mice have five pairs of mammary glands, two pairs of mammary glands in the inguinal region and three pairs in their thoracic area which are not present in cattle. In addition, each mammary gland has only one teat opening and one primary duct, similar to this cows. This mouse mastitis model also provides the suitable tool for research in control of bovine intramammary infections. Observations in mice model mastitis concerning bacterial counts, neutrophil numbers and histological changes are similar to those in cows and allows interaction of the organism with the host cells and immune components (Chandler, 1970b).

1.3.1 Differential Leukocyte Count (DLC)

Neutrophils predominate in the cellular infilterate at sites of acute inflammation and are activated by signals generated by activated endothelium and/or molecules emitted

from the source of inflammation (Albeda *et al*., 1994). The relative percentage of lymphocytes decreases significantly and the relative percentage of neutrophils increases after bacterial infection in challenged groups (Sharma *et al*., 2013).

1.3.2 C-reactive protein (CRP)

C-reactive protein (CRP) is an acute-phase protein consisting of five identical polypeptide chains that form a five-membered ring with a molecular weight of 120,000 Daltons. C-reactive protein belongs to the pentraxin family of proteins and it is synthesized in the liver by hepatocytes. During acute and chronic inflammatory conditions like bacterial, viral, or fungal infections its levels rise dramatically up to 50,000 fold. CRP is used mainly as a marker of inflammation. Measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatments (Pepys and Hirchfield, 2003). In healthy individuals CRP concentrations in blood are extremely low but may be fast increased after induction of inûammatory response associated with infections, autoimmune and cardiovascular diseases, as well as sepsis and cancer. Elevated blood CRP levels have been widely used as an ideal marker of inûammation (Van Leeuwen and Van Rijswijk, 1994). The CRP concentrations in cattle with bovine mastitis was more than 10-fold the concentration in healthy cattle. During an investigation of a dairy farm, the serum levels of CRP in cows with mastitis were much higher (1083 ± 93 ng/mL) than those of healthy cows (82 ± 66 ng/mg) (Schrodl *et al*. 1995). CRP concentrations increase rapidly in serum and often exceed the reference range by 1000 times or more (Mortensen, 2001).CRP is mainly used as a marker of inflammation. In the present study, the semi-qualitative analysis of C- reactive protein was performed in the blood serum of experimental animals. It rises above its normal limits within 6 hours, and peaks at 48 hours; the plasma half-life of CRP is about 19 hours and is constant under all conditions of health and disease, so that the sole determinant of circulating CRP concentration is the synthesis rate (Vigushin *et al*., 1993). CRP values can never be diagnostic on their own and can only be interpreted at the bedside, in full knowledge of all other clinical and pathological results. Mouse CRP is a trace protein whose concentration increases only modestly, to a maximum

of about 2 mg/ml, during the acute-phase response. In vivo work on CRP function has largely been confined to passive administration of exogenous, heterologous CRP or to mice transgenic for rabbit or human CRP (Pepys and Hirschfield, 2003).

1.3.3 Interleukin-12 p40 (IL-12p40)

Interleukin-12 is produced by activated antigen-presenting cells (dendritic cells, macrophages) (Dorman and Holland, 2000) in response to intracellular pathogens. It promotes the development of Th1 responses and is a powerful inducer of IFNγ production by T and NK cell (Newport *et al*., 2007). IL-12 is a heterodimeric cytokine composed of two disulfide-bonded subunits, p35 and p40 which has important regulatory effects on T cells and natural killer (NK) cells is important mediators of inflammatory disease (Gately *et al*., 1998). IL-12 plays a major role in the host defense against predominantly intracellular microbial infection and cancer. IL-12 may also be involved in allergic disorders and autoimmune diseases showing its immunologic regulatory properties.

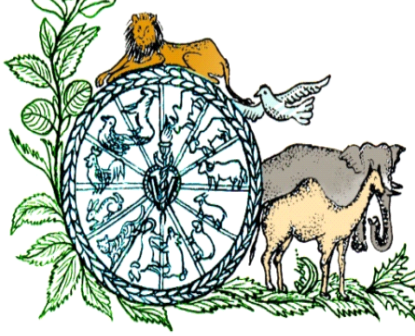
1.4 Study location

West Singhbhum is located in the eastern part of India and is one of the 24 districts in Jharkhand richest in terms of plant diversity; traditional healing systems are still popular here. People are poor and economically they depend on cattle grazing, agriculture and use of natural resources. Therefore, the traditional medicines are the preferred by such people and people rely on indigenous medicinal plant for basic health care treatment. So, far no systematic ethnobotanical survey has been made in this area and this is the first report on the medicinal plants used by the local traditional healers for treatment of bovine mastitis in West Singhbhum district of Jharkhand. The current study was aimed to explore and document the indigenous knowledge of plants and to evaluate the importance of medicinal plants used in local healthcare system.

Hence, the present study has been planned with following objectives:

- ***To collect the scattered knowledge of Ethnoveterinary practices used for treatment of bovine mastitis in tribal areas of the district West Singhbhum, Jharkhand.***
- ***To document the collected indigenous practices in original as well as with modifications.***
- ***To Validate selected indigenous practices by PRA (Participatory Rural Appraisal) and QuIK (Quantification of Indigenous knowledge) method.***
- ***In vitro and in vivo validation of the most frequently used biodynamic agents against intramammary infections.***

Review of Literature



2 REVIEW OF LITERATURE

2.1 Ethnoveterinary medicine

Ethnoveterinary medicine is a traditional knowledge, folk beliefs, skills, methods and practices used for the treatment of livestock ailments (Mathias-Mundy and McCorkle, 1989; Tabuti *et al*., 2003). Farmers in various developing regions still use medicinal plants for treatment of livestock diseases due to lack of access to veterinarians and price of modern medicines. Ethnoveterinary traditional practices are continuing since farmers believe that medicinal plants are more efficacious for treatment of livestock ailments than modern medicines (Harun-or-Rashid *et al*., 2010). Traditional veterinary medicine knowledge may be lost due to rapid socioeconomic, environmental, technological changes and as a result of the loss of cultural heritage under the guise of civilization (Mathias-Mundy and McCorkle, 1989; Nfi *et al*., 2001).Conventional veterinary services have been playing a paramount role in the control and prophylaxis of livestock diseases over the last three decades in the country but they cannot yet deliver complete coverage in preventive and curative health care practices of livestock due to the high cost of drugs and equipment, an erratic supply of drugs, logistical problems and inadequate labour (Sori *et al*., 2004). The knowledge is transferred from generation to generation through the word of mouth with great secrecy. This suggests documenting and conserving through ethnoveterinary studies before it is lost forever. The documentation of indigenous traditional knowledge on the medicinal uses of plants has provided many important drugs of modern day (Balick and Cox, 1996).

Indigenous Traditional Knowledge (ITK) is a community based functional knowledge system, developed, preserved and refined by generations of people through continuous interaction, observation and experimentation with their surrounding environment. It is a dynamic system, ever charming, adopting and adjusting to the local situations and has close links with the culture, civilization and religious practices of the communities (Pushpangadan *et al*., 2002). The communities have developed the indigenous knowledge system to conserve and utilize the biological diversity of their surroundings. The recognition of the creativity of the traditional communities is essential for the conservation of biodiversity as well as conservation of intellectual diversity (Jena, 2007).

Medicine is the science and art of healing. Early records on medicine have been discovered from ancient Egyptian medicine, Babylonian medicine, ayurvedic medicine (in the Indian subcontinent), classical Chinese medicine (predecessor to the modern traditional Chinese Medicine) and ancient Greek medicine and Roman medicine. The famous "Code of Hammurabi" in 1800 B.C. concerning the fees veterinarians could charge for treatment of cattle and donkeys (Schwabe, 1984). During the reign of King Ashoka between 269 and 232 B.C. in the Rock Edict II suggests the first known veterinary hospitals of the world (Somvanshi, 2006). Indus Valley civilization is one of the foremost contributors in the history of development of veterinary science and animal husbandry (Somvanshi, 2006). Later on, China, Egypt and Arabia developed into the centers of veterinary practices. Ethnoveterinary not only includes treatments like herbal preparations, surgical interventions, indigenous vaccination but people's ideas about disease causation, transmission including zoonosis, religious and cultural attitudes, beliefs and rituals, husbandry operations for overall good health, people's perception of relationship between environment and animal healthcare and social organizations having know how about animal health care (McCorkle,1986).

McCorkle *et al*. (1996) stated, "In theory all systems may work; in practice, all have successes and failures with some systems scoring much higher in particular areas of medicine depending on the social, cultural and economic context in that they are applied". In fact, it would be naive to think that either ethno or Western science alone is likely to provide a sufficient solution for all development problems present today. So, more research on alternative

methods of disease prevention and control is recommended (Gueye, 2002). Rural communities developed mixed systems of production of crops and the rearing of animals are combined with income generating off-farm activities. Rich and efficient ethno-veterinary traditions exist in the villages and comprise of beliefs, knowledge, practices and skills pertaining to health care and management of livestock. Many herders and farmers manage and treat their animals including poultry species and companion animals without any inputs that cost money (Hooft, 1997) Livestock healers are usually farmers themselves. Healers learn their craft from a parent or other relative. Some healers are remarkably professional. They had their own ready-made drug preparations such as herbal tablets, decoctions and ointments for treating different types of diseases (Padmakumar, 1997). Some healers may be 'generalists' offering treatments for many ailments of most animal species or 'specialized' offering certain types of treatment as herbal medicines, prepare a single drug from several plants the combination of that is specific for each disease, firing (cauterisation), massage, castration or spiritual/religious interventions. Villages having professional healers and their treatments are often more easily available and sometimes the farmers are saved from adulterated costly drugs (Padmakumar, 1997). Modern sector has failed to recognize the importance of healers as health care providers, despite the fact that stock raisers consult such healers many times and that some kind of professional advice is required even when conventional veterinary choices are available (Mathias, 2004). In ancient cultures people developed their own herbal pharmacopoeias based on information gained through experience and in our today's scientific pharmacopoeia much of the information on scientific medicine is derived from those herbal pharmacopoeias (Kim, 2005). Prior to the discovery of organic chemistry in the 19th century, 80% of all medicines were obtained from plant materials.

2.2 Bovine Mastitis

Bovine mastitis is a major disease that causes economic losses to dairy industry and decreased milk production with reproductive disorders of dairy cows (Seegers *et al*., 2003; Santos *et al*., 2004). Bovine mastitis is a highly prevalent disease in dairy cattle, and one of the most important diseases affecting the world's dairy industry; it places a heavy economic burden on milk producers all over the world (Miller *et al*., 1993). Mastitis is one of the most severe

diseases in dairy cow despite the progress made in improving general udder health in recent years. Epidemiological studies have revealed that the main pathogens are bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Streptococcus dysgalactiae* and *Streptococcus agalactiae* and so on. *Staphylococcus aureus* causes contagious and mostly chronic mastitis in cattle worldwide (Barkema *et al*., 2006). The disease is associated with reduced milk quality and high economic loss (Gröhn *et al*., 2004; Halasa *et al*., 2007) and is therefore a key problem for the dairy industry. *Staphylococcus aureus* is one of the major causes of subclinical, clinical, recurrent and chronic mastitis in dairy cattle. These infections are commonly treated with antimicrobial agents. It is known fact that based on various epidemiological studies reveals a moderate correlation exists in between the antimicrobial susceptibility of *S. aureus* and its bacteriological cure therapy of the patient (Sol *et al*., 2000). Increasing evidence indicates that biofilm formation by *S. aureus* at the site of infection is responsible for different antibacterial resistance of *S. aureus* against bovine mastitis (Cucarella *et al*., 2004; Oliveira *et al*., 2006).

E. coli is environmental pathogens, which is originated from the organic matter of cowshed (Hogan and Smith, 2003). *E. coli* infects the udder via the teat canal (Eberhart, 1984). An environment contaminated with faeces is the main source of mastitis causing *E. coli* bacteria (Linton *et al*., 1979). Poor barn design and management of dairy cows may also provide the cows with predisposing factors for coliform mastitis (Ward *et al*., 2002; Krause *et al*., 2003). The severity and outcome of *E. coli* mastitis can vary between cows in the same herd and in the same individual during different lactation stages. The observed systemic and local clinical signs results from the acute-phase reaction, which is the response of the host to any tissue injury caused by trauma or inflammation (Van Miert, 1995). Physiological stress around parturition and metabolic diseases, for instance, may be associated with increased susceptibility of periparturient cows to coliform mastitis (Burvenich *et al*., 2003). The clinical characteristics and outcome of *E. coli* mastitis vary from mild mastitis, where cows have only local signs in the udder and the duration of the infection is short, to very severe or even fatal forms (Burvenich *et al*., 2003). The environmental streptococci, *Streptococcus uberis* in particular, have been isolated from bedding materials, soil, rumen, feces, vulva, lips, nares, mammary gland and teats (Epperson *et al*., 1993).

2.3 PRA (Participatory Rural Appraisal)

In 1970s it was realized by the researchers that there should be some data collection methods to produce reliable and cost-effective information helpful in designing rural development projects in developing countries (Chambers, 1983). The formal methods were not suited to understand the problems of the poorest people living in rural communities. In response to this situation, alternative systems of inquiry were developed. These systems included Rapid Rural Appraisal (RRA) and Participatory Rural Appraisal (PRA). These systems are based on involvement of local people in defining their problems, analyzing them and finding the solution. So less informal survey methods started replacing formal survey methods. Less formal tools used in social anthropology and experience of indigenous knowledge systems began to merge with the field testing of informal interviewing, visualization and other methods (Chambers, 1994). Lack of budgets or insufficient funding for development projects were the main factors favoring the use of faster approaches than the time consuming anthropological fieldwork methods.Cost-effective ways to learn and assess the problems of rural people and data collection led to development of Rapid Rural Appraisal and Participatory Appraisal Techniques (Catley, 1999). Rapid Rural Appraisal is a collection of cost effective ways to learn about the researched situation, needs and initiatives of rural people and to collect relevant data for planning projects (Waters-Bayer and Bayer, 1994). Tools include interviewing, diagramming, scoring and ranking, mapping and visualization. Rapid Rural Appraisal aims for faster collection of better quality data and speedier analysis than given by conventional questionnaires (Waters-Bayer and Bayer, 1994). Of these tools interviews were the most important group of methods because they were used alone but also complemented and formed the basis for other tools. Interviews were generally informal and semi structured. For documentation of EVM particularly both conventional and participatory methods are used either separately or in combination. In veterinary medicine questionnaire surveys have been widely used to collect information. Important considerations while using questionnaire are target population and sampling method, questionnaire design, administration and quality control (Catley, 1999). Participatory Rural Appraisal is considered one of the popular and effective approaches to gather information in rural areas. This approach was developed in early 1990s with considerable shift in paradigm

from top-down to bottom-up approach.The basic concept of PRA is to learn from rural people. Participatory Rural Appraisal represents a body of qualitative methods that emphasize the use of indigenous or local knowledge and that can be adapted to virtually any research situation. Participatory Rural Appraisal is carried out by multidisciplinary team consisting of a leader and a few core members who act as facilitators to assist local people to elicit and record their own knowledge using techniques that involve a minimum outsider interference or involvement. (Langill, 1999). PRA is intended to enable local communities to conduct their own analysis and to plan and take action; PRA involves project staff learning together with villagers about the village. The aim of PRA is to help strengthen the capacity of villagers to plan, make decisions, and to take action towards improving their own situation.

2.4 Quantification of Indigenous knowledge methodology

Validation of ITKs has been done through quantification of Indigenous knowledge methodology (QuIK) method by some identified persons who were experienced in particular ITKs, developed by DeVilliers (1996). The basic premise of this method is that farmers know and understand the environment in which they farm and that answers to many questions can be found in the collective experience of the farming community and doing informal experiments over years. It can be used to unpack the practices of successful farmers, so that information can be disseminated to a wider group of farmers. QuIK methodology represents a rapid and relatively cheap way to elicit indigenous traditional knowledge.

2.5 Herbal Plants

Researchers and pharmaceutical entrepreneurs agree that ethnobotanically derived compounds have greater activity than compounds derived from random screening and therefore a greater potential for novel products developed (Cox and Balick, 1996; Flaster, 1996). Plants that are employed in traditional medicines worldwide are two to five times more likely to test out as pharmacologically active than those randomly sampled (Mathias *et al*., 1996; Natarajan and Iyer, 2000). Consequently there is a growing interest in traditional uses of plants for health care among different communities especially in the developing countries.The therapeutic potential of medicinal herbs is dependent on pharmacologically active organic

compounds e.g. alkaloids, glycosides, flavonoids etc., which are the second metabolic products of plant cells (Heinrich *et al*., 2004; Poschenrieder *et al*., 2008; Capasso, 2003). Since ancient times plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings as well as livestock (Lulekal *et al*., 2008; Devi *et al*., 2009). Medicinal plants are integral component of EVM. Medicinal plants used by traditional healers are mostly found to be effective (McCorkle and Green, 1998). Governments are taking interest to increase investment in complementary and alternative medicine for achieving the goal of cost reduction in health services and the medicinal plants are cost-saving replacements of commercial drugs (Mathias, 2004; Kim, 2005). Farmers and pastoralists have a long history of the use of traditional medicine. The make up the bulk of the population and have a deep knowledge of their environment (Nfi *et al*., 2001), medicinal plants for several centuries have been widely used as a primary source of prevention and control of livestock diseases. Curative properties of herbs for human and animal ailments have long been known and are documented in ancient manuscripts in India (Takhar, 2004). Medicinal plants are cost-saving replacements of commercial drugs (Mathias, 2004). Not only the resource poor farmers but the intensive production units use the medicinal plants (Mathias, 2004). Market and public demand of medicinal plants has been increased and there is great risk that many medicinal plants today, face either extinction or loss of genetic diversity (Kudi, 2003).

2.6 Mouse Models

A mouse model of infectious mastitis was first described by Chandler (1970a). Since then, this model has been used to assess the physiopathology of *S. aureus* intramammary infections (Reid *et al*., 1976; Anderson, 1976, 1978; Chandler *et al*., 1980; Brouillette *et al*., 2003a) and the role of potential bacterial virulence factors (Jonsson *et al*., 1985). The protection conferred by immunization (Mamo *et al*., 2000; Gomez *et al*., 1998) and the effect of antibiotic administration (Bramley and Foster, 1990; Sanchez *et al*., 1994) on *S. aureus* intramammary infections were also studied using the mouse mastitis model. Despite the work done to this day, this model has never been characterized or optimized for antibacterial compound efficacy studies. The present work aims to extensively characterize the mouse mastitis model (inoculum size, time of infection, dissemination of bacteria, polymorphonuclear cell infiltration, destruction

of tissue, herbal and antibiotic treatment) while using well known and commercially available strains of mice and bacterium. With the mouse model of mastitis, mainly intramammary infections caused by *S. aureus* have been studied (Brouillette and Malouin, 2005). The mouse model of *Staphylococcus aureus* mastitis has been used to study bacterial pathogenesis and its treatment (Mamo *et al*., 1991). *S. aureus* intramammary infection in the mouse is similar to that in the cow in that it induces inflammation and PMN infiltration of mammary tissue. However, in contrast with cows, milk collection from mice for somatic cell count analysis is a difficult and time consuming procedure. In order to use mouse mammary gland sections for the evaluation of tissue alterations and inflammation during mastitis, an efficient method for quantification of PMN infiltration is necessary.

2.7 Plant parts used and mode of preparation

For preparation of drugs, parts of the plants that are mostly utilized are roots, barks, wood, leaves, stems, flowers, fruits, juices, resins, latex, grains, buds, bulbs and seeds (Dilshad *et al*., 2008). Medicine is mostly administered to the animals orally as decoctions, liquid in that the plants have been steeped, vaccination, suppositories, through smoke, vapours, massage, intranasally or applied topically on the skin or as a bathe in skin problems (Muhammad *et al*., 2005). Extraction using solvents with different polarities can be used to roughly fractionate a complex mixture of compounds. The Soxhlet process (Houghton and Raman, 1998) is useful for the exhaustive extraction of plant material with a single solvent at a time.

2.8 Honey

Honey is one of the most important natural products widely used not only in food industry, but also in medicine due to its antibacterial properties. Volatile fraction of honey contains more than 150 organic compounds (Castro-Vazquez *et al*., 2003). There have been many reports of the beneficial effects of honey used as a topical treatment for a wide range of wounds, ulcers and abscesses (Phuapradit and Saropala, 1992). Efem (1988) reported that infected wounds and ulcers became sterile within one week of topical application of honey. Honey has also been shown to be effective in treating a variety of ulcers (Greenwood, 1993). It can also provide a viscous barrier preventing infection and fluid loss of the wound, can

absorb oedema fluid and can act as an anti-inflammatory by relieving pain (Subrahmanyam, 1991). Molan (1992) collated a comprehensive list in his review on the antibacterial activity of honey. Honey has been shown to be effective against Gram positive and Gram negative organisms, aerobic and anaerobic bacteria, as well as inhibiting spore germination of *Bacillus cereus* (El-Sukhon *et al*., 1994). The major acid present in honey is gluconic acid (Stinson *et al*., 1960) produced by glucose oxidase. Another compound produced by this reaction is hydrogen peroxide. Hydrogen peroxide is known to have antimicrobial properties and much evidence exists to suggest that it is this compound which confers antimicrobial activity to honey (Molan, 1992). Radwan *et al*. (1984) found honeys to retain antimicrobial activity against some bacterial and fungal species. Honey is likely to be most effective in treating localised infections like mastitis where a reasonably high concentration of honey can be achieved (Willix *et al.,* 1992). Honey contains antibacterial compounds that are effective in inhibiting or killing a broad spectrum of bacteria (Mavric *et al*., 2008).

2.9 Differential Leukocyte count

White blood cell counts (TLC) and differentials (DLC) can vary substantially between strains, sexes, and ages of mice (Bannerman, 1983), illustrating the importance of consistency during an experiment. Comparative changes in blood and mammary mononuclear leucocyte population during the periparturient period are well documented (Yang *et al*., 1997). Following invasion of mammary gland by pathogens, macrophages and epithelial cells release chemoattractants; this triggers the migration of leukocytes, mainly PMNs from blood towards mammary gland (Leitner *et al*., 2000). The presence of functional PMNs is crucial to the host defense against bacterial pathogens (Pappe *et al*., 2003).

2.10 C-Reactive Protein (CRP)

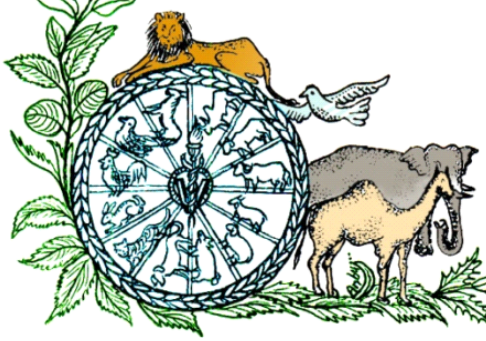
C-reactive protein (CRP) is a 224 residue protein with a monomer molecular mass of 25106 Da. C-reactive protein belongs to acute phase proteins, whose serum level may increase several dozen times due to inflammation, infection or other tissue damaging factors (Lloyd *et al.,* 2006). CRP is mainly used as a marker of inflammation. Hepatocytes are the main source of C-reactive protein; however, it may also be synthesized at extrahepatic sites like adipocytes

and sites of tissue injury (Dehghan, 2007). It is thought to bind with phosphocholine on microbes, thus initiating recognition and phagocytosis of damaged cells (Pepys and Hirschfield, 2003). C-reactive protein plays important role in innate immunity as early defense system against infections. It assists in complement binding to foreign and damaged cells and enhances their phagocytosis by macrophages, expressing the receptors for CRP. CRP rises upto 50,000-fold in acute inflammations, such as mastitis. It rises above its normal limits within 6 hours, and peaks at 48 hours. The half-life of CRP is constant and therefore its level is mainly determined by rate of production. Estimating and charting C-reactive protein values is useful in determining disease progress or the effectiveness of treatments. The concentration of C-reactive protein was shown to increase in bovine serum and milk during mastitis (Schroedl *et al.,* 2003).

2.11 IL-12p40

IL-12 is a 75 kDa heterodimer (IL12p70) comprised of independently regulated disulûde-linked 40 kDa (p40) and 35 kDa (p35) subunits (Murphy *et al*., 2000). The p40 subunit, as a monomer (IL12p40) or homodimer (IL12 (p40)2), can also bind to the IL-12 receptor resulting in interactions that antagonize IL-12p70 binding both in mice (Ha *et al*., 2002) and humans (Ling *et al*., 1995). An enzyme-linked immunosorbent assay (ELISA), provide reliability at the expense of direct measurement of IL-12 bioactivity. Mature dendritic cells (DCs) are some of the most proliûc producers of IL-12 and play a critical role in regulating the immune response (Hart, 1997). The production of IL-12p70, IL-12p40, and IL-12 (p40)2 by mature DC is highly dependent on the cells cumulative exposure to inûammatory mediators during differentiation and maturation (Ebner *et al*., 2001) and thus provide a link between the peripheral tissues and lymphoid organs. A diagnostic method like ELISA seems necessary for monitoring the IL-12p40 in health and mastitis.

Materials and Methods



3 MATERIALS AND METHODS

3.1 Location of the study

West Singhbhum district form the Southern part of the newly created Jharkhand State and is the largest district in the State. The district spread over 21° 58' and 23° 36' north latitude and 85° 0' and 86° 54' east longitudes. The district is situated at a height of 244 Meter above the sea level and has an area of 5351.41 Sq. Kilometers. The district is bounded on the north by the district of Ranchi, on the east by Saraikela-Kharsawan district, on the south by Keonjhar, Mayurbhanj and Sundargarh districts of Orissa and on the west by the district of Gumla and Sundargarh (in Orissa). West Singhbhum remains with 15 blocks and two administrative Sub-divisions (Chaibasa and Chakradharpur). The district is full of hills alternating with valleys, steep mountains, and deep forests on the mountain slopes. The district contains one of the best sal forests and the SARANDA (seven hundred hills) forest area is known world over. The majority of the population in West Singhbhum district is tribal population. The scheduled tribes residing in the district are - Asur, Baiga, Banjara, Bathudi, Bedia, Binhia, Birhor, Gond, Gorait, Ho, Kumali, Kharia, Kharwar, Khond, Kisen, Chero, Chik Baraik, Lohara, Mahli Munda, Oraon, Parhaiya, Kora, Korwa, Santhal, Sawar, Bhumij etc.

The indigenous traditional veterinary practices (TIVPs) collected from 12 tribal villages of two subdivisions namely, Chaibasa and Chakradharpur of West Singhbhum district of Jharkhand state, Chotanagpur region.

3.2 Identification of the resource person

For collection of traditional indigenous veterinary practices (TIVP) being used by livestock owners of that area, the identification of resource persons/farmers and traditional veterinary healers (TVH) about their practices was done by means of Nomination method and Participatory Rural Appraisal (PRA) technique. During selection, some of the important considerations were:

i. Those that belongs to the particular area
ii. Those having good experience of TIVP
iii. Those whom people in general seek advice on animal husbandry matters
iv. Those whom people in their villages or adjacent areas considered as the knowledgeable in TIVP in animal husbandry
v. Those who are respected in their peer group and people listen to them

The local healers generally do not reveal the TIVP and it is very difficult to get their knowledge. Interviewing the actual users could solve this problem to some extent. Hence, both the local healers as well as the actual users were the respondents for this study. An interview schedule was developed for both categories of respondents.

Following the above method, 30 resource persons (local healers) for veterinary treatment and 320 experienced farmers using these traditional indigenous veterinary practices were selected.

3.3 Collection and screening of TIVP from resource persons

A detailed discussion with the identified resource persons were conducted on mastitis for which TIVP practiced in animal treatment in the respective area. The information was also taken from various secondary sources as well as through observation. Information was also collected from veterinary surgeons.

In order to confirm the plant materials or other ingredients or local household substances used to heal mastitis in animals were requested to show and observations were made to know the actual procedure of application or mixing of different ingredients.

Table 1: Village selected and interviewed

Chaibasa	Chakradharpur
1. Guira	1. Chandri
2. Lupunggutu	2. Padampur
3. Patahatu	3. Toklo
4. Purna chaibasa	4. Potka
5. Tonto	5. Otar
6. Pancho	6. Asantaliya

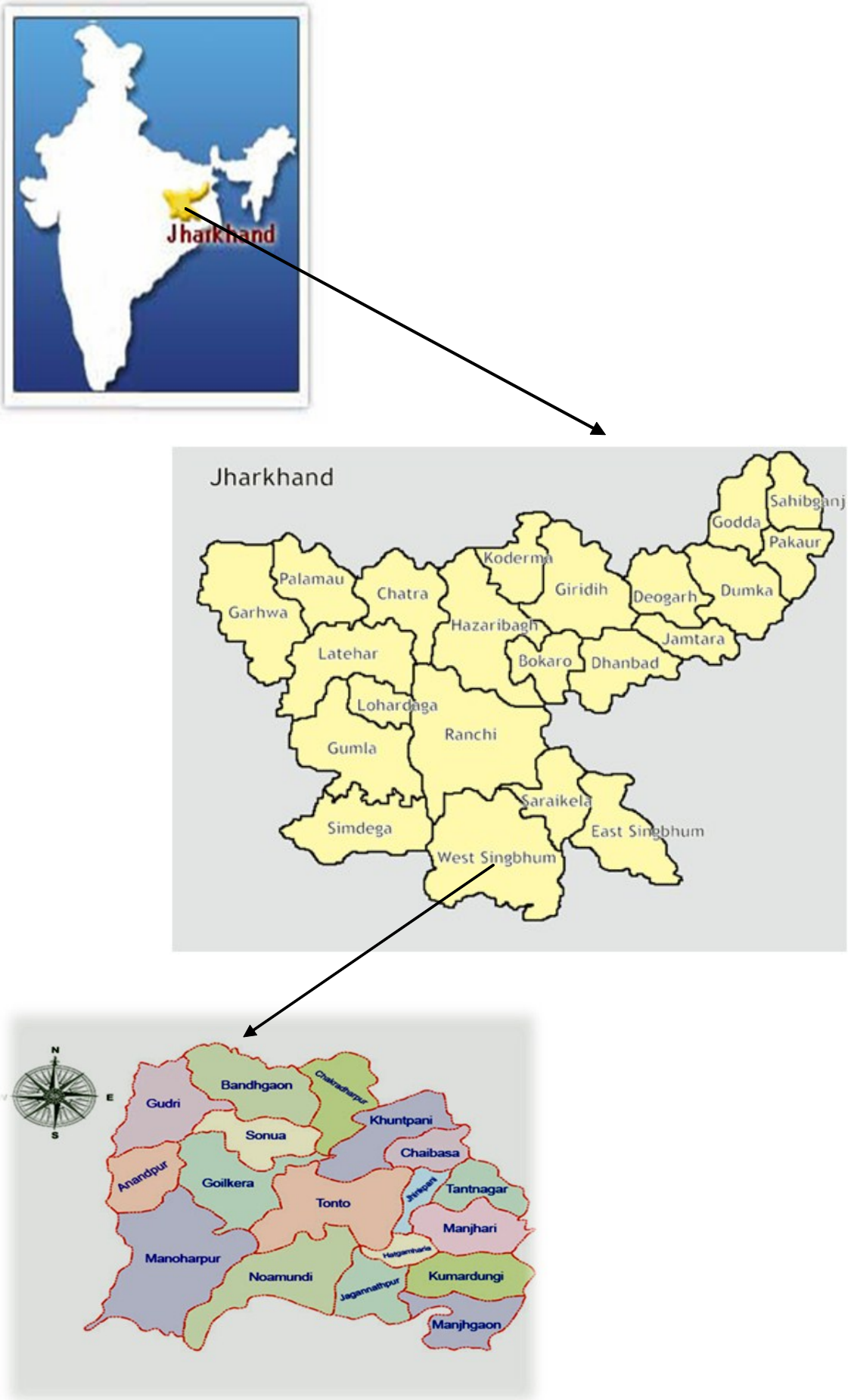


Fig 1: Location map of study area

3.4 Documentation of indigenous traditional knowledge

Documentation of TIVP was done and having the following information

1. Key ingredients
2. Method of preparation
3. Method of application
4. Doses

3.5 Variables and their measurements

The relevant variables for the present study were selected after having a scanning of the available literatures, consultation with scientists.

3.5.1 Independent Variables

3.5.1.1 Personal and socio Economic characteristic

3.5.1.1.1 Age

This refers to the chronological age (in years) of the respondents rounded to the nearest whole number at the time of interview. It was ascertained by direct questioning was also rounded to the nearest whole number. Respondents were categorized into following three categories.

i. Young (<47 years)
ii. Middle (47-64 years)
iii. Old (>64 years)

3.5.1.1.2 Caste

A caste is a social category whose members are assigned a permanent status within a given social hierarchy and whose contacts are restricted accordingly (Lundbarg *et al*., 1968). It was ascertained by direct question. Respondents were categorized into 4 groups.

i. Scheduled caste (SC)
ii. Scheduled tribes (ST)
iii. Other backward classes (OBC)
iv. General

3.5.1.1.3 Family type

It refers to the type of family i.e. whether joint or nuclear family.

3.5.1.1.4 Herd size

It refers to the number of total animal's viz. cattle, buffaloes, goats, sheep and other farm animals, a respondent had at the time of enquiry. On the basis of the obtained results the respondents were categorized into three categories.

i. Small
ii. Medium
iii. Large

3.5.1.1.5 Land holding

It refers to actual areas of land in bighas, being cultivated by the respondents. It was measured by direct questioning. The respondents were categorized into three groups.

i. Small
ii. Medium
iii. Large

3.5.1.1.6 House

Respondents were categorized into 2 groups and were asked by direct questioning

i. One storeyed mud
ii. Cemented house

3.5.1.1.7 Education

It refers to the functional literacy and/or academic qualification of the respondent which was ascertained by direct questioning. The respondents were assigned scores according to their education level as;

i. Illiterate
ii. Primary
iii. Middle
iv. Intermediate

3.5.2 Communication characterstic

3.5.2.1 Extension agency contact

It refers to the extent of contact made by respondents with the change agents such as Livestock Extension workers, A.I. officers, Veterinary Surgeon, Village Level Workers etc. a structure schedule was developed to measure the frequency of visit. The respondents were scored on a three point continuum scale namely often, occasionally and never. The scoring system was 2, 1, 0 respectively, for the three points on the scale. The respondents were categorized into three groups.

i. Low
ii. Medium
iii. High

3.5.2.2 Mass media exposure

It refers to the degree of the mass media viz. radio, T.V., Newspaper etc. This was measured by using a schedule developed for this purpose. Scores 2, 1, 0 were assigned for often, occasionally and never. According to the obtained score the respondents were categorized into the following three categories.

i. Low
ii. Medium
iii. High

3.5.2.3 Informal information sources used

It refers to the degree of utilization of informal information sources viz. family members, neighbors, friends etc. This was measured by developing a schedule for this purpose. Score 4, 3, 2, 1, 0 were assigned for everyday, most often, often, sometimes and never respectively. According to the obtained score the respondents were categorized into the following three categories.

i. Low
ii. Medium
iii. High

3.6 Data collection

Data were collected regarding profile of respondents (Traditional veterinary healers and traditional farmers) with the help of interview schedule developed for the study. For documentation of indigenous traditional knowledge, data were collected from the respondents with help of interview schedule developed for this study. Later on, data were collected from traditional farmers for selected Indigenous Traditional Knowledge (ITKs) to measure extent of awareness and use of ITKs with Modern veterinary drug (MVD). Data were collected from both traditional veterinary healers and farmers to obtain information about sources and channels of filtration and transfer of knowledge. At last, data were collected from the traditional farmers for validation studies by Quantification of Indigenous Knowledge method.

3.7 Validation

3.7.1 Field Validation

3.7.1.1 Quantification of Indigenous Knowledge (QuIK)

Validation of ITKs has been done through QuIK method by some identified persons who were experienced in particular TIVP using the method QuIK developed by De Villiers (1996). The basic premise of this method is that farmers know and understand the environment in which they farm and that answers too many questions can be found in the collective experience of the farming community and doing informal experiments over years. It can be used to unpack the practices of successful farmers, so that information can be disseminated to a wider group of farmers. QuIK methodology represents a rapid and relatively cheap way to elicit indigenous traditional knowledge.

Farmers who were experienced in the particular ITKs taken for validation were chosen. In QuIK, PRA tool based on matrix ranking is combined with an interview schedule to elicit numerical data from experienced farmers. The matrix was designed through preliminary discussions with farmers and was then obtained as part of a systematic process to obtain quantitative data. Quantitative data obtained through preliminary discussions with farmers and respondents were asked to weigh the ITKs in comparison with modern veterinary drugs for its performance on different criteria, such as effectiveness (How many animals are cured?), cost

effectiveness, quickness in healing, easy in preparation, side effect and availability. For the validation of different ITKs the respondents were asked to put the pebbles out of 10 in each block of matrix. Unlike others, in case of side effects, the greater value of stone indicates fewer side effects. The same matrix was used to interview a number of farmers and the data from each farmer was treated as an independent result.

3.7.1.2 Fidelity level (FL %)

The percentage of informants claiming the use of a certain plant for the same major purpose for identification of most frequently used plant in that area was analyzed using quantitative index like FL%,

$$FL\ (\%) = (Np / N) \times 100$$

Where Np is the number of informants citing the use of the plant for a particular illness and N is the total number of informants citing the species for any illness. Generally, high FLs are obtained for plants for which almost all use reports refer to the same way of using it, whereas low FLs are obtained for plants that are used for many different purposes (Srithi *et al*., 2009).

3.7.2 Laboratory Validation

3.7.2.1 *In vitro* validation of ITKs

3.7.2.1.1 Selection of most frequently used herbs selected from questionnaire and preparation of extracts

i. Collection of plant material

A total of six herbal products were selected on the basis of QuIK methodology and Fidelity level**.** Fresh root of *Glycyrrhiza glabra* (*G. glabra*), bark of *Listea monopetala* (*L.monopetala*), *Bombax ceiba* (*B. ceiba*) and *Butea monosperma* (*B. monosperma*), fruits of *Terminalia bellerica* (*T. bellerica*) and *Piper longum* (*P. longum*) collected from West Singhbhum district, were authenticated from Department of Botany, Bareilly College, Bareilly (Voucher specimen no. 2014064199a, 2014064199b, 2014064199c, 2014064199d, 2014064199e and 2014064199f). These plant materials were washed thoroughly with distilled water, shade dried for seven days and crushed and pulverized by mechanical grinder to make a coarse powder, the powder was used for extract preparation.

ii. Aqueous extract

Air dried material (50 gm) of Fresh root of *Glycyrrhiza glabra*, bark of *Listea monopetala*, *Bombex ceiba* and *Butea monosperma*, fruits of *Terminalia bellerica* and *Piper longum* were finely pulverized and extracted with distilled water in soxhlet apparatus upto 4 cycles (Nang *et al*., 2007). Then filter the extract using whatmann filter paper no. 1, the extract was then collected and dried carefully below 40°C, taking care not to char the extract. It was weighed in its completely dry form and the yield was found to be 10 gm for *Glycyrrhiza glabra*, 3 gram for for *Listea monopetala,* 6 gram for *Bombex ceiba*, 7 gram for *Butea monosperma*, 11 gram for *Terminalia bellerica*, 9 gram for *Piper longum* were finally reconstituted in phosphate buffer saline (PBS) and filtered through membrane filter (0.45 µm pore size) and dried, weighed and stored in clean 10 ml capacity plastic jars with tightly closed lids.

iii. Methanolic extract

About 50 gm of powdered root of *Glycyrrhiza glabra*, bark of *Listea monopetala*, *Bombex ceiba* and *Butea monosperma*, fruits of *Terminalia bellerica* and *Piper longum* was taken in a clean flat-bottomed glass container and percolated with 250 ml of methanol. The container with its content was sealed and kept for 15 days with occasional sealing and stirring. The mixture was then filtered through whatmann filter paper no. 1. The filtrate thus obtained kept at 40°C for evaporation of methanol. The yield was found to be 8 gm for *Glycyrrhiza glabra*, 11 gram for *Listea monopetala*, 7 gram for *Bombex ceiba* and *Butea monosperma*, 9 gram for *Terminalia bellerica*, 10 gram for *Piper longum* is finally reconstituted in phosphate buffer saline (PBS) and filtered through membrane filter (0.45µm pore size) and dried, weighed and stored in clean 10 ml capacity plastic jars with tightly closed lids.

iv. Hydromethanolic extract

About 50 gm of powdered root of *Glycyrrhiza glabra*, bark of *Listea monopetala*, *Bombex ceiba* and *Butea monosperma*, fruits of *Terminalia bellerica* and *Piper longum* was taken in a clean flat-bottomed glass container and percolated with mixture of methanol

and distilled water in the ratio of 70:30. The container with its content was sealed and kept for 15 days with occasional sealing and stirring. The mixture was then filtered through filter paper. The filtrate thus obtained kept at 40°C for evaporation of methanol and water. The yield was found to be 7 gm for *Glycyrrhiza glabra*, 2 gram for *Listea monopetala*, 6 gram for *Bombex ceiba* and *Butea monosperma*, 8 gram for *Terminalia bellerica* and *Piper longum*, finally reconstituted in phosphate buffer saline (PBS) and filtered through membrane filter (0.45 µm pore size) and dried, weighed and stored in clean 10 ml capacity plastic jars with tightly closed lids.

3.7.2.1.2 Preliminary phytochemical screening

The plant materials were screened for the presence of different classes of secondary metabolites including alkaloids, glycosides, saponins, tannins, sterols and flavonoids, using previously described methods.

3.7.2.1.2.1 Physical characterstics

The physical characterstics of the extracts were analysed by the method described by (Khandelwal, 2005).

3.7.2.1.2.2 Qualitative Chemical investigation

All the selected methanolic extracts of best three herbs (*Glycyrrhiza glabra, Listea monopetala* and *Piper longum*) were tested for the presence of phytoconstituents (Khandelwal, 2005).

3.7.2.1.2.2.1 Test for detection of Flavonoids

- **Sodium hydroxide test**

About 5 mg of the compounds was dissolved in water, warmed and filtered, 10% aqueous sodium hydroxide was added to 2 ml of this solution. This produces a yellow colouration, a change in colour from yellow to colourless on addition of dilute HCl was an indication for the presence of flavonoids.

3.7.2.1.2.2.2 Test for Saponins

- **Foam test**

A few mg of the test residue was taken in a test tube and shaken vigorously with small amount of sodium bicarbonate and water. If stable, characteristic honey comb like froth was obtained, saponins were present.

3.7.2.1.2.2.3 Test for Tannins

- **Potassium dichromate test**

The test residue of each extract was taken separately in water, warmed & filtered. Tests were carried out with the filterate by adding a solution of potassium dichromate in test filterate, dark colour was obtained, showing presence of tannins.

3.7.2.1.2.2.4 Test for alkaloids

- **Wagner's test**

Few mg of residue of each extract was taken separately in 5 ml of 1.5% v/v hydrochloric acid and filtered, when few drops of wagner's reagent (1.27 gram of iodine and 2 gram of potassium iodide were dissolved in 5 ml of water and the solution was diluted to 100 ml with water) were added to the test filterate, a brown colour precipitate was formed indicating the presence of alkaloids.

3.7.2.1.2.2.5 Test for glycosides

- **Legal test**

The test is employed for digitoxase contain glycosides. The few mg of residue of each extract was dissolved in pyridine. When sodium nitroprusside solution was added to it, pink or red colour was produced.

3.7.2.1.2.2.6 Test for Sterols

- **Salkowski test**

Few mg of residue of each extract was taken in 2 ml of chloroform and in it 2 ml of concentrated sulfuric acid was added from the side of test tube. The test tube was shaken for

few minutes. The development of red colour in chloroform layer indicates the presence of sterols.

3.7.2.1.3 Collection of Milk Samples for isolation of pathogenic organisms

The isolation of pathogenic organisms was done from the milk of cows suffering from subclinical mastitis at the organized dairy farms of IVRI. The lactating cows were screened by conducting their physical and clinical examination as per the method of Schalm *et al*. (1971) for exclusion of abnormal shape, size and consistency as udder possessing lumps, oedema, atrophy, fibrosis and placement of teats, blind teats and indurations. The cows with sub clinical cases of bovine mastitis were diagnosed by California Mastitis Test (CMT) of milk samples collected from all the quarters of the udder and interpretation was done as per the method of Schalm and Noorlander (1957).

3.7.2.1.3 .1 California mastitis test (CMT)

CMT testing of the milk samples was done during milking. About 5 ml milk was collected from each quarter in CMT paddle. An equal volume of CMT reagent was added to all the 4 parts of paddle containing milk samples to visualize any change in colour and consistency in terms of gel formation and its viscosity. The grading of the reaction is done by the intensity of gel formation and colour changes as given in Table 2.

3.7.2.1.3.2 Isolation of all the major pathogens ie Coagulase Negative *Staphylococci*, Coagulase Positive *Staphylococci*, *Streptococci* and *E. coli* from mastitic milk samples

Isolation and identification of pathogenic organism from the mastitis milk samples was done as per the standard procedure (Griffin *et al.,* 1977). Microorganisms were initially identified on the basis of colony morphology and odour on 5% blood agar as per Cruikshank (1962) and later by gram's staining and growth on selective media. Each milk sample was streaked directly on 5% bovine blood agar used as a general purpose growth media for the cultivation, selective isolation, and differentiation of organisms responsible for mastitis in dairy populations. *Staphylococcus aureus* (*S. aureus*) will appear round and shiny with golden-yellow colonies demonstrating a zone of beta-hemolysis while *Streptococcus* species will demonstrate alpha, beta, or non-hemolytic patterns and are often white to gray in color (National Mastitis Council,

1999). From Blood agar 3-5 colonies were taken and streaked on selective media and differential media like Mannitol salt agar and Baird Parker agar for identification of *Staphylococcus sps.* For identification of *Streptococcus agalactiae* (*St. agalactiae*) Blood agar and Edward media and for identification of *Escherichia coli* (*E. coli*) Eosine methylene blue agar (Appendix VII), then all the plates were incubated at 37°C for 24-48 h, before discarding the plate as negative. Characteristic colonies were selected and pure isolates were transferred on to nutrient agar slants in duplicates. After incubation and morphological check for purity, the slant cultures were assigned a number and stored at 4^{o}C. One out of the two slant cultures was used for further biochemical characterization.

3.7.2.1.3.3 Biochemical characterization

Pathogens were identified by standard biochemical kits (HiStaph™, HiStrep™ and Hi *E. coli*™ identification kit HiMedia, Mumbai).

3.7.2.2 Antimicrobial susceptibility test

i. McFarland turbidity standard

To standardize the inoculum density for a susceptibility test, $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standards was used. A 0.5 McFarland standard was prepared as described in NCCLS (1997). One percent v/v solution of sulfuric acid was prepared by adding 1 ml of concentrated sulfuric acid to 99 ml of water and mixed well. A 1.175% w/v solution of barium chloride was prepared by dissolving 2.35 g of dehydrated barium chloride ($BaCl_2.H_2O$) in 200 ml of distilled water. To make the turbidity standard, 0.5 ml of the barium chloride solution was added to 1% 99.5 ml sulfuric acid solution and mixed well. A small volume of those turbid solutions was transferred to a screw-capped tube of the same type as used for preparing the control inoculum and stored in the dark at room temperature. 0.5 McFarland standard represents 1.5×10^8 bacteria/ml of the suspension.

ii. Antibiotic disc sensitivity test (ABST) of the isolated strains and selected extracts of herbs

Antibiotic sensitivity of all the major pathogens ie Coagulase Negative *Staphylococci*, Coagulase Positive *Staphylococci*, *Streptococci* and *E. coli* were carried out by disc diffusion method of Kirby *et al.* (1968). Briefly, each culture was inoculated into sterilized nutrient

Table 2: Point scores of CMT with description

CMT grade	Description	Point score
Negative (N)	No change	0
Trace (T)	Slime formation which disappeared with continous movement of paddle	1
Weak (+)	Distinct slime but no gel formation, slight purplish colour	2
Distinct Positive (++)	Viscous with gel formation which adhered to the margin of the cup with colour changes	3
Strong Positive (+++)	The gel forms convex projection, the gel did not dislodge after swirling movement of paddle	4 and 5

broth incubated at 37° C for 12-18 h. All tests were performed on Mueller-Hinton agar. The surface was lightly and uniformly inoculated by bacterial suspension having visually equivalent turbidity to 0.5 McFarland standards by L shape rod. The standard antimicrobial discs of Amoxycillin (5 µg/disc), Ampicillin (10 µg/disc), Cefotaxime (30 µg/disc), Chloramphenicol 30 µg/disc), Ciprofloxacin (5 µg/disc), Cotrimazole (25 µg/disc), Enrofloxacin (5 µg/disc), Erythrromycin (15 µg/disc), Gentamicin (10 µg/disc), Penicillin G (10 IU /disc) and Streptomycin (10 µg/disc) (Hi-Media Ltd.) and sterile discs were impregnated in 25 µl of the prepared herbal extracts (with concentration of 2 mg /25 µl, 4 mg/25 µl, 6 mg/25 µl and 8 mg/25 µl) were aseptically placed on the dried surface of agar. The inoculated plates were placed at 4°C for 2 hours and thereafter the plates were incubated at 37°C for 24 hours. The plates were read by taking measurement of zone of inhibition in millimeters. All the tests were performed in duplicates.

iii. MIC of all the selected extracts of herb will be done by double dilution method (NCCLS, 1998)

Inoculum for the MIC test was prepared by taking at least three to five well-isolated colonies of the *St. agalactiae* from an agar plate culture. The top of each colony was touched with a sterile loop and the growth was transferred into a tube containing 4 to 5 ml of normal saline. The broth culture was incubated at 37°C until it achieved the turbidity of the 0.5 McFarland standards (usually 2 to 6 hours). This results in a suspension containing approximately 1.5×10^8 cfu/ml. The turbidity of the actively growing broth culture was adjusted with sterile broth to obtain turbidity comparable to that of the 0.5 McFarland standards. MIC is the lowest concentration of an antimicrobials that inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents (Andrews, 2001). Any, turbidity, after overnight incubation connoting at least 10 fold increase in bacterial density. For oral drenching in mice, the dosage (in mg/kg) was taken to be nearly 20 times the MIC.

Dosage (mg/kg) of herbal extracts = MIC× 20

3.7.2.3 Dose determination of the biodynamic agents

a) Glycyrrhiza glabra

The methanolic extract of *G. glabra* showed no turbidity at a concentration of 1.56 mg/ml and above, indicating its antibacterial activity at 1.56 mg/ml against *St. agalactiae* at these concentration. The dose of *G. glabra has* taken to be nearly 20 times of MIC i.e. 31.20 mg/kg. Each mouse weighing about 30 grams on an average was given 0.936 mg/day after dissolving in sterile PBS and drenched orally, daily.

b) Listea monopetala

The methanolic extract of *L. monopetala* showed no turbidity at a concentration of 1.56 mg/ml and above, indicating its antibacterial activity at 1.56 mg/ml against *St. agalactiae* at these concentration. The dose of *L. monopetala* has taken to be nearly 20 times of MIC i.e. 31.20 mg/kg. Each mouse weighing about 30 grams on an average was given 0.936 mg/day after dissolving in sterile PBS and drenched orally, daily.

c) Piper longum

The methanolic extract of *P. longum* showed no turbidity at a concentration of 6.25 mg/ml and above, indicating its antibacterial activity at 6.25 mg/ml against *St. agalactiae* at these concentration. The dose of *P. longum* has taken to be nearly 20 times of MIC i.e. 125 mg/kg. Each mouse weighing about 30 grams on an average was given 3.75 mg/day after dissolving in sterile PBS and drenched orally, daily.

3.7.2.2 *In vivo* validation of ITKs

3.7.3 Experimental animals

i. Mice

Adult healthy Swiss albino female mice, weighing not less than 30 g were procured from the vivarium, Laboratory Animal Resource (LAR) Section, Indian Veterinary Research Institute, Izatnagar immediately after weaning from their first lactation. Thereafter the mice were kept in the Divisional Animal Shed under ideal conditions of management. The trial was

carried out in the Animal shed, Division of Medicine, Indian Veterinary Research Institute, Izatnagar during the year 2013-2014 in lactating mice challenged with *St. agalactiae* by intramammary route which were compared with lactating healthy mice.

ii. Institutional Animal Ethics Committee Approval

The experimental design and research plan along with animal handling and disposal procedure were approved from Institutional Animal Ethics Committee of IVRI with IAEC-Reg No. 196/RO/bc/2000/CPCSEA, 01.06.2000, IAEC Approval No. 1-53/2012-13.

3.7.4 Therapeutic trial in mice experimental mastitis model

3.7.4.1 Experimental trial in mouse mastitis model

The experimental trial was conducted in 7 days post litter lactating mice using right- 4 (R-4) and left- 4 (L-4) inguinal mammary glands. Forty-two 7 days post litter lactating mice were divided into 7 groups comprising of 6 animals each. Group I (negative control), Group II (positive control), Group III (Methanolic root extract of *Glycyrrhiza glabra* + Honey drenched orally), Group IV (Methanolic fruit extract of *Piper longum* + Honey drenched orally), Group V (Methanolic bark extract of *Listea monopetala* + Honey drenched orally), Group VI (combination of methanolic extract of root of *Glycyrrhiza glabra,* fruit of *Piper longum,* bark of *Listea monopetala* + Honey treated) and group VII (Ciprofloxacin treated) animals were used for the trial. Antibiotic was selected on the basis of antibiotic sensitivity test. The cases of mastitis were screened by visual and microbiological examinations (Sridevi, 2005). The cases were further screened using clinical examination score card (Table 3).

3.7.4.2 Oral drenching of the biodynamic agents in mice

The biodynamic agents were drenched to the mice of the respective groups with the help of 18 gauge needles fitted to small 1 ml syringes after reconstitution with PBS, in case of methanolic root extract of *Glycyrrhiza glabra* dose was (31.20 mg/kg), for methanolic fruit extract of *Piper longum* dose was (125 mg/kg) and for methanolic bark extract of *Listea monopetala* dose was (31.20 mg/kg). Honey were given at the dose rate of (0.2 ml twice daily orally). Extract were drenched 7 days prior and 7 days post inoculation (Table 4). Honey was only used as vehicle in administration of biodynamic agents.

3.7.4.3 Antibiotic administration in mice

The Ciprofloxacin was selected after scrupulous antibiotic sensitivity testing of *Streptococcus agalactiae* against various antibiotics in vitro as per method described by Kirby *et al*. (1968). It was given orally to the mice @ 5 mg/ kg body wt orally for 7 days after post inoculation (Table 4).

3.7.4.4 Selection of pathogenic *St. agalactiae* strain for intramammary challenge of mice

St. agalactiae obtained from cow number 17 with sub clinical mastitis was found suitable both bacteriology and biochemically. The strain was sub cultured continuously to keep it viable and used as the challenge strain. Strict laboratory conditions were exercised to disable any sort of contamination of the isolate under all circumstances.

3.7.4.5 Dose of *St. agalactiae* for intramammary challege

Dose of bacteria required for intramammary inoculation was calculated by surface viable count methodnas described by Miles and Mishra (1938). Streptococcal suspension was serially diluted by adding 1 ml of suspension to 9 ml of sterile NSS. Three plates were made for statistical reasons so that an average of at least three counts could be made. A 20 µl of the appropriate dilution equal to 10^{-5} was dropped from a height of 2.5 cm onto the surface of agar over an area of 1.5 to 2.0 cm and the drop allowed spreading naturally. Touching the surface of agar with pipette was avoided. The plates were left upright on the bench to dry before inversion and incubation at 37°C for 18 hours. The plates were observed for growth, high concentrations giving a confluent growth over the area of the drop. Colonies were counted in the sector where the highest number of full size discrete colonies were observed (usually sector containing between 2-20 colonies were counted). Following equation is used to calculate the number of colony forming units (CFU) per ml from original sample:

CFU per ml = Average number of colonies for a dilution x 50 x dilution factor.

The numerical factor "50" in the formula accounts for the number of bacteria in 1 ml as we take only 20 µl (0.02 ml) of inoculated broth initially in the test.

Table 3: Clinical Examination Score Card

Score	General Demeanor	Swelling No change	Discoloration	Histopathology
0	Normal	No swelling of mammary glands	No. change of colour	Normal alveoli containing milk
1	Dull	Mild swelling of mammary glands	Reddish discolouration of mammary glands	Mild inflammatory reaction with neutrophils
2	Mild depression	Moderate swelling of mammary glands	Reddish discolouration of mammary glands with exudation of blood stained exudate	Moderate inflammatory reaction with neutrophils and lymphocytes
3	Severe depression/ Recumbent/ General illness	Severe swelling of mammary glands	Bluish discolouration of mammary glands	Severe inflammatory reaction with neutrophils, MNCs lymphocytes and necrotic changes
4	Dead	Swollen and hardened mammary glands	Discolouration of mammary glands with presence/ exudation of pus	Severe inflammatory reaction with neutrophils, MNCs lymphocytes, necrotic changes and abscess formation

Table 4: Plan of therapeutic trial

Group	No. of Mice	Treatment	Dosage	Route	Interval	Duration
I	6	Negative control	-	-	-	-
II	6	Positive control	-	-	-	-
III	6	Methanolic extract of *Glycyrrhiza glabra* + Honey	0.936 mg/day	Orally	Twice	14 days
IV	6	Methanolic extract of *Piper longum* + Honey	3.75 mg/day	Orally	Twice	14 days
V	6	Methanolic extract of *Listea monopetala* + Honey	0.936 mg/day	Orally	Twice	14 days
VI	6	Combination of Methanolic extract of *Glycyrrhiza glabra, Piper longum, Listea monopetala* + Honey	1.87 mg/day	Orally	Twice	14 days
VII	6	Ciprofloxacin	5 mg/kg BW	Orally	Twice	7 days

*The dose was determined as per the MIC of the extracts.
**Herbal extracts were drenched 7 days prior and 7 days post inoculation

Following surface viable count method the dose of 18 hour incubated broth culture of *St. agalactiae* taken for intramammary challenge in mice was 5×10^5 bacteria in each mammary gland.

3.7.4.6 Challenge of Mice with Pathogenic *St. agalactiae*

The skin of the medial left and right hindleg, inguinal skin region and the skin around the nipples of all the animals was sampled for bacteriological examination as described by Hermans *et al*. (1999) before the onset of experiment. After one week of parturition the mice were challenged with the pathogenic *St. agalactiae* following the methodology of Chandler (1970a). The mice were anaesthetized with ketamine (100 mg/ kg) and xylazine (10 mg/kg). Abdomen of mice was disinfected with 70 % ethanol. Teat tips of left and right fourth mammary glands (L4 and R4, respectively) were removed aspectically with the help of sterilized scissor and 0.1 ml of 18 hr incubated culture of target organism *St. agalactiae* containing approximately 10^8 colony forming units (CFU) was injected intramammary using 26 gauge blunt needle (Trigo *et al.,* 2009).

3.7.4.7 Collection of blood from mice for hematological parameters

Blood was collected from each mouse by puncturing the retrobulbar venous plexus through the inner eye canthus using heparinised microcapillary tubes taking care not to injure the ocular structures (Van Herck *et al*., 1992b). Blood was collected in tubes without any anticoagulant for separation of serum to evaluate C-reactive protein (CRP) and Intrleukin-12 p40 (IL-12 p40). Simultaneously thin blood smears were made on microslides and air dried and immediately fixed with methanol and stained with (1:10) Geimsa stain (Appendix VII) for a period of 30 minutes for differential leucocyte count (DLC) estimation.

3.7.4.8 Differential leukocyte count (DLC)

Differential leukocyte count (DLC) was performed in blood of experimental animals at 0 h, 48 h, 96 h and 144 h. Methanol fixed blood smears were stained with diluted (1:10) Giemsa stain (Appendix VII) for 40 minutes. The smears were washed with PBS and dried. These blood smears were then examined microscopically under oil immersion. Neutrophils, lymphocytes, eosinophils, monocytes and basophils were identified, counted and results were expressed in percentage (Jain, 1986).

3.7.4.9 C-reactive protein (CRP)

The C-reactive protein (CRP) was evaluated in the serum samples of experimental animals using a standard kit by Span diagnostic limited, CRP (Latex Agglutination Method) at 0 h, 24 h, 48 h and 96 h, as per the instructions of the manufacturer.

3.7.4.10 IL-12 P40

The IL-12 P40 was in the serum samples of experimental animals using a standard kit RayBio,Mouse IL-12 (P40/P70) ELISA Kit at 0 h and 96 h.

Reagents required:

1. IL-12 (P40/P70) Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-Mouse IL-12 (P40/P70).
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution.
3. Standards (Item C): 2 vials, recombinant IL-12 (P40/P70).
4. Assay Diluent A (Item D): 30 ml of animal serum with 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.
5. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For Standard/Sample (cell culture medium) diluent.
6. Detection Antibody IL-12 (P40/P70) (Item F): 2 vial of biotinylated anti-Mouse IL-12 (P40/P70) (each vial is enough to assay half microplate).
7. HRP-Streptavidin Concentrate (Item G): 200 µl of 400x concentrated HRP-conjugated streptavidin.
8. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.
9. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.

Procedure:

Prepare all reagents, samples and standards as instructed. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature or over night at 4°C. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

3.8 Collection of mammary glands for histopathological and microbiological examination

3.8.1 Collection of Mammary Glands

All mice from each treated and untreated group were sacrificed at 144 hours post challenge. Whole mount was prepared from mouse mammary glands by the method described by Rasmussen *et al.* (2000). Mice were given chloroform inhalation to produce light anaesthesia. The mouse was then placed in a recumbent position, ventral side up, on a cork board using a suitable method of restraint and was swabbed with 70% ethanol. A ventral midline inverted Y incision was made beginning midway between nipples 4 and 5 just above the genital area toward the thorax (about 2.5 cm) and laterally to each leg (about 1.5 cm) between the nipples. The skin flaps, with mammary glands attached, were carefully separated from the peritoneum with a blunt-edged instrument. The free edge of each skin flap was secured to the cork board, thereby exposing the adherent mammary glands. Mammary glands were carefully excised from the skin flap by using blunt scissors. Mammary gland was spread onto the centre of a pre-labeled glass slide without any air bubbles and hair. Margins of the gland was extended as wide as possible and allowed to air dry for about 30 seconds to enhance the adherence. Any change in the gross appearance of glands such as discoloration and swelling was recorded and noted. R4 glands were placed directly into bottle containing formal saline 10 % (v/v) and were processed for histopathological and histochemical examination. L4 glands were directly processed for bacterial isolation.

3.8.2 Histopathological examination

Histopathological examination was performed in healthy, infected untreated and treated mice. Parraffin blocks containing mammary tissue samples were cut into sections of approximately 3-5 μ size and were processed for histopathological examination by H&E staining as per the standard method described by Culling (1963).

3.8.2.1 Staining (H & E)

Staining of mammary gland whole mount was performed with Mayer's haematoxylin and eosin stain. The staining protocol followed is given below briefly:

1. From Carnoy's fixative, the glands were rehydrated by passing through descending grades of alcohol and brought to water.
2. The slides were kept in the staining solution for few hours to overnight depending on the thickness of the gland.
3. Rinsed with distilled water to remove the excess stain.
4. The glands were dehydrated by passing through ascending grades of alcohol followed by clearing with xylene-I and II.
5. The glands were permanently mounted without air bubbles using DPX mountant.

3.8.3 Demonstration of bacteria in mammary tissue

Mammary tissue sections of experimental animals (96 h interval) were stained by Gram's and Geimsa staining as per the methodology described by Culling (1963).

3.8.4 Bacterial isolation and Bacterial counts of L4 mammary glands

L4 mammary gland from mice from each group was triturated with 1 ml sterile chilled NSS in a sterile pestle and mortar on ice. A 10 microlitre of suspension was spread over on to 5 % Blood agar or nutrient agar plates by L shape and after incubation for 18-24 hours at 37^0 C the colonies were counted as per the method described by Griffin *et al.* (1977).

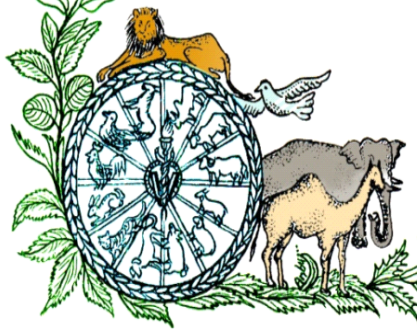
3.9 Media and chemicals

The media and reagents used for the study have been listed in the Appendix VII

3.10 Statistical analysis

The data collected on each parameter was analyzed by standard statistical methods. Level of significance was set ($P<0.05$) by applying Friedman test for total clinical scores. Level of significance was set ($P<0.05$) by applying two way ANOVA for DLC and level of significance was set ($P<0.05$) by applying one way ANOVA for validation of herbal plants and ITKs. (SPSS, 17.0 Software).

Results



4 RESULTS

4.1. Profile of the respondents (traditional healers) (N=30) in the selected villages of West Singhbhum district, Jharkhand

4.1.1 Personal and Socio Economic Characteristic

4.1.1.1 Age

Majority of respondents (53.34%) were falling under middle age (47-64 years) followed by 33.34% and 13.34% in young age (less than 47 years) and old age (more than 64 years) respectively.

4.1.1.2 Caste

Study revealed that 16.67% of the respondents belonged to scheduled caste (SC), 46.67% scheduled tribe (ST), 30.00% other backward class (OBC) and remaining 6.67% to general community.

4.1.1.3 Family type

Majority of respondents 83.34% belonged to joint family, whereas remaining 16.67% belonged to nuclear family.

4.1.1.4 Herd size

Study revealed that majority of respondents 36.67% had medium herd size, 56.67% of the respondents had small herd size. Remaining 6.67% farmers had large herd size.

4.1.1.5 Land holding

Study manifested that majority of the respondents have small land holding (73.34%) followed by (20.00%) medium land holders and high land holders (6.67%).

4.1.1.6 Types of house

Table 5 reveals that majority of respondents (86.67%) had the one storeyed mud house followed by cemented house (13.34%).

4.1.1.7 Education

Study indicated that majority of respondents (36.67%) were illiterate, followed by primary (26.67%), middle (23.34%), and intermediate (13.34%). These findings clearly indicate that the area had low education status.

4.1.2 Communication characteristic

4.1.2.1 Extension agency contact

Study revealed that 50.00% of the respondents had medium extension agency followed by low (30.00%) and high (20.00%) respectively.

4.1.2.2 Mass media exposure

The distribution of respondents according to their mass media exposure in table 5, shows that as many as 40.00% of them had a medium level of mass media exposure, followed by low level 33.34% and high level with 23.34% .

4.1.2.3 Informal information sources used

The distribution of respondents according to their informal information sources used revealed that as many as 46.67% of respondents comes under medium category followed by low (30.00%) and high (23.34%) category respectively (Table 5).

Table 5: Profile of the respondents (traditional healers) (N=30) in the selected villages of West Singhbhum district, Jharkhand

Sl No.	Variables	Categories	Frequency	Percentage
Personal and socio-economic characterstic				
1	Age	Young (<47 years)	10	33.34
		Middle (47-64 years)	16	53.34
		Old(>64 years)	4	13.34
2	Caste	SC	5	16.67
		ST	14	46.67
		OBC	9	30.00
		General	2	6.67
3	Family Type	Joint	25	83.34
		Nuclear	5	16.67
4	Herd Size	Small (<2.1532 cattle unit)	17	56.67
		Medium(2.1532-6.709 cattle unit)	11	36.67
		Large(>6.709 cattle unit)	2	6.67
5	Land Holdings	Small (< 2.3268 bigha)	22	73.34
		Medium (2.3268-6.7842 bigha)	6	20.00
		High (>6.7842 bigha)	2	6.67
6	House	One storeyed mud	26	86.67
		Cemented house	4	13.34
7	Education level	Illiterate	11	36.67
		Primary	8	26.67
		Middle	7	23.34
		Intermediate	4	13.34
Communication characterstic				
8	Extension agency contact	Low (<1.3212)	9	30.00
		Medium (1.3212-4.4564)	15	50.00
		High(>4.4564)	6	20.00
9	Mass media exposure	Low(<1.4363)	10	33.34
		Medium(1.4363-6.4525)	12	40.00
		High(>6.4525)	7	23.34
10	Informal information sources used			
		Low(<3.2908)	9	30.00
		Medium(3.2908- 6.8202)	14	46.67
		High(>6.8202)	7	23.34

4.2 Profile of the respondents (traditional farmers) (N=320) in the selected villages of West Singhbhum district, Jharkhand

4.2.1 Personal and Socio Economic Characteristic

4.2.1.1 Age

Majority of respondents (55.63%) were falling under middle age group (47-64 years) followed by 34.37% and 10.00% in young age (less than 47 years) and old age (more than 64 years) respectively.

4.2.1.2 Caste

10.94% of the respondents belonged to SC, 44.38% ST, 41.56% OBC and remaining 3.12% to general community.

4.2.1.3 Family type

Majority of respondents 74.37% belonged to joint family whereas remaining 28.75% belonged to nuclear family.

4.2.1.4 Herd size

Study revealed that majority of respondents had small herd size 47.81 %. 41.25% of the respondents had medium herd size. Remaining 10.94% farmers had large herd size.

4.2.1.5 Land holding

Study manifested that majority of the respondents (51.87%) were small land holders followed by medium (35.00%) and high land holders (16.25%).

4.2.1.6 Type of house

Table 6 revealed that majority of respondents (75.62%) had the one storeyed mud house followed by cemented house (27.50%).

4.2.1.7 Education

Study indicated that majority of respondents were primary (35.00%), followed by, middle (25.00%), intermediate (22.50%) and (17.50%) were illiterate. These findings clearly indicate that the low education profile of the respondents in the study area.

4.2.2 Communication characteristic

4.2.2.1 Extension agency contact

Study revealed that 48.44% of the respondents had medium extension agency contact followed by low (39.37%) and high (12.19%) respectively.

4.2.2.2 Mass media exposure

The distribution of respondents according to their mass media exposure in table 6, showed that as many as 50.62% of them had a medium level of mass media exposure, followed by low level 42.81% and high level with 6.57%.

4.2.2.3 Informal information sources used

The distribution of respondents according to their informal information sources used in table 6 revealed that as many as 49.37% of respondents comes under medium category followed by high (41.25%) and low (9.37%) category respectively.

4.3 Documentation of traditional methods

4.3.1 Feeding and watering practices

i. Animals were maintained on paddy straw. Some of the respondents provided 2-3 kg of paddy straw/animal, whereas some of them provided 3-4 kg of paddy straw/animal.
ii. All the cattle's were allowed to graze on green grasses only because of the lack of fodder.
iii. Lactating cows were provided with some vegetable waste and rice husk.
iv. The source of water for the animal was ponds, tube well and well.
v. No treatment of water was done before giving it to the animals.
vi. Ad libitum water was being given to the animals.

4.3.2 Breeding practices

i. Heat detection was done by observing the following behaviors- mounting, frequent bellowing or bleating, mucus discharge from vagina, restlessness, frequent urination, loss of appetite.

Table 6: Profile of the respondents (traditional farmers) (N=320) in the selected villages of West Singhbhum district, Jharkhand

Sl No.	Variables	Categories	Frequency	Percentage
Personal and socio-economic characterstic				
1	Age	Young (<47 years)	110	34.37
		Middle (47-64 years)	178	55.63
		Old(>64 years)	32	10.00
2	Caste	SC	35	10.94
		ST	142	44.38
		OBC	133	41.56
		General	10	3.12
3	Family Type	Joint	238	74.37
		Nuclear	92	28.75
4	Herd Size	Small (<2.1532 cattle unit)	153	47.81
		Medium(2.1532-6.709 cattle unit)	132	41.25
		Large(>6.709 cattle unit)	35	10.94
5	Land Holdings	Small (< 2.3268 bigha)	166	51.87
		Medium (2.3268-6.7842 bigha)	112	35.00
		High (>6.7842 bigha)	52	16.25
6	House	One storeyed mud	242	75.62
		Cemented house	88	27.50
7	Education level	Illiterate	56	17.50
		Primary	112	35.00
		Middle	80	25.00
		Intermediate	72	22.50
Communication characterstic				
8	Extension agency contact	Low (<1.3212)	126	39.37
		Medium (1.3212-4.4564)	155	48.44
		High(>4.4564)	39	12.19
9	Mass media exposure	Low(<1.4363)	137	42.81
		Medium(1.4363-6.4525)	162	50.62
		High(>6.4525)	21	6.57
10	**Informal information sources used**			
		Low(<3.2908)	132	41.25
		Medium(3.2908- 6.8202)	158	49.37
		High(>6.8202)	30	9.37

ii. Method of service: Both natural mating and artificial insemination method were in practice.

iii. Recognition of pregnancy: Pregnancy was detected by observing the following behaviors, erection of teat, distention of abdomen, absence of estrus.

iv. During parturition animals were kept under close observation.

4.3.3 Management of newborn calves

Following practices is in vogue regarding management of newborn calves.

i. Removal of mucus from nostril and buccal cavity.

ii. Wiping and drying the body of new born with a piece of clean, dry cloth.

iii. Cutting of naval cord with blade or thread

iv. Keeping the newborn in close vicinity to dam

v. Provision of bedding materials like straw, gunny bags for calf

vi. Allowing the newborn to suck the colostrum.

4.3.4 Milking

Full hand milking was practiced by farmer. There was two fixed time for milking morning and evening.

4.3.5 General health care practices

4.3.5.1 Detection of sick animals

Animals who shows the following behaviors were detected as sick by the farmers

i. Inappetance

ii. Absence of rumination

iii. Dry muzzle

iv. Tear in eyes

4.3.5.2 Detection of mastitis

i. Inflamed udder

ii. Change in colour of milk

iii. Animal taking less feed

iv. Sometimes fever

4.4 Extent of awareness and use of indigenous technical practices in original form with modifications if any with modern veterinary drug (MVD).

The extent of awareness and use of indigenous technical practices and their parallel scientific technology (MVD) are presented and discussed below. The Indigenous Traditional Knowledge (ITKs) which were taken for validation was taken into account (Table 7).

4.4.1 Different ITKs for the treatment of Bovine mastitis

1. ITK1- *Cucurma longa* root 30 gram grinded with jaggery along with 200 gram of *Brassica compestres* seed and administered orally for 5 days. Out of 320 respondents 46.85% of the respondents were aware of this practice; however 53.12% of the respondents disagree to this practice and supports MVD for the treatment of mastitis.

2. ITK 2- About 48.75% of the respondents were agree to give orally, 200 gram *Terminalia bellerica* fruit grinded with 100 gram *Nigella sativa* seed and 40 gram *Foenicum vulgare* seeds crushed together boil in water till one fourth of remaining volume and drenched orally for 8 days. Whereas in case of MVD, 51.25% of the respondents were disagree to this practice.

3. ITK 3- About 48.12% of the respondents were agree to administer *Allium sativum* 200 gram, *Oryza sativa* 250 gram, *Zinziber officinale* 200 gram grinded with sugar and drenched orally for 5 days, whereas 51.87% of the respondents in MVD class disagree to this practice..

4. ITK 4- The paste of the bark of *Listea monopetala*, leaves of *Azardirachta indica*, bark of *Butea monosperma* and bark of *Bombex ceiba* and applied over the inflamed part of the udder for 7 days, about 47.18% of the respondents were aware of it, whereas 52.81% of the same respondents were agreed for MVD.

5. ITK 5- Administration of 100 gram of *Piper longum*, 50 gram of *Phyllanthus embellica* grinded along with leaves of *Ocimum sanctum* and sugar and drenched orally for 5 days was common practice. About 47.81% of the respondents were aware of it, whereas 52.18% of the same respondents were agreeing for MVD.

Table 7: Extent of awareness and use of indigenous traditional knowledge with modern veterinary drug (MVD) (N=320) in the selected villages of West Singhbhum district, Jharkhand

Indigenous practices	No. of respondents		Parallel Scientific Technology	No. of respondents	
	Farmers	Percentage		Farmers	Percentage
ITK 1 *Cucurma longa* root 30 gram grinded with jaggery along with 200 gram of *Brassica compestris* seed and administered orally for 5 days	170	46.85	MVD	150	53.12
ITK 2 200 gram *Terminalia bellerica* fruit grinded along with 100 gram *Nigella sativa* seed and 40 gram *Foenicum vulgare* seeds crushed together boil in water upto one fourth of original volume and drenched orally for 8 days	164	48.75	MVD	156	51.25
ITK3 *Allium sativum* 200 gram, *Oryza sativa* 250 gram, *Zinziber officina*le 200 gram grinded with sugar and drenched orally for 5 days	166	48.12	MVD	154	51.87
ITK 4 Make the paste of the bark of *Listea monopetala*, *Butea monosperma*, *Bombex ceiba* and leaves of *Azardirachta indica* and applied over the inflamed part of the udder for 7 days	169	47.18	MVD	151	52.81
ITK 5 Administration of 100 gram of *Piper longum*, 50 gram of *Phyllanthus emblica* grinded along with leaves of *Ocimum sanctum* and sugar and drenched orally for 5 days was common practice	167	47.81	MVD	153	52.18
ITK6 A paste is made from the root of *Glycyrrhiza glabra*, *Nardostachys Jatamansi*, *Nigella sativa* along with ghee and applied on udder and teat	153	52.18	MVD	167	47.18

Table 7: Contd...

Indigenous practices	No. of respondents		Parallel Scientific Technology	No. of respondents	
	Farmers	Percentage		Farmers	Percentage
ITK 7 50 gram of *Embelia ribes*, 60 gram powdered roots of *Withania Somnifera* along with 100 gram seeds of *Trachyspermum ammi* and 30 gram of *Elleteria cardamomum* boiled together with 5 liters of water until the remaining liquid is one quarter of the original volume and drenched orally for 7 days	169	47.18	MVD	151	52.81
ITK 8 China Clay was blended with water or *Brassica compestres* oil, or a combination of both and topically applied over udder three four times until healing	176	45.00	MVD	144	55.00
ITK 9 A paste is made from leaves of *Luffa cylindrica*, *Lawsonia inermis*, *Punica granatum* and applied over affected part of the udder for 7 days. It was very effective in case of gangrenous mastitis	163	49.06	MVD	157	50.93
ITK10 Leaves paste of *Centella asiatica*, *Datura alba* and *Tabernaemontana divaricata* mixed with oil of *Semicarpus anacardium* and applied as a poultice in case of udder infection	172	46.25	MVD	148	53.75

6. ITK 6- A paste is made from the root of *Glycyrrhiza glabra*, *Nardostachys Jatamansi*, *Nigella sativa* along with ghee and applied on udder and teat. About 52.18% of the respondents were aware of it, whereas 47.81% of the same respondents have chosen MVD.

7. ITK 7- 50 gram of *Embelia ribes*, 60 gram powdered roots of *Withania Somnifera* along with 100 gram seeds of *Trachyspermum ammi* and 30 gram of *Elleteria cardamomum* boiled together with 5 liters of water until the remaining liquid is one quarter of the original volume and drenched orally for 7 days. About 47.18% of the respondents were aware of it, whereas 52.81% of the same respondents used MVD.

8. ITK 8- China Clay was blended with water or *Brassica compestres* oil, or a combination of both and topically applied over udder three four times until healing, about 45.00% of the respondents were aware of it, whereas 55.00% of the same respondents used MVD.

9. ITK 9- A paste is made from leaves of *Luffa cylindrica*, *Lawsonia inermis*, *Punica granatum* and applied over affected part of the udder for 7 days. It was very effective in case of gangrenous mastitis. About 49.06% of the respondents were aware of it, whereas 50.93% of the same respondents used MVD.

10. ITK 10- 46.25% respondents were aware of applying leaves paste of *Centella asiatica*, *Datura alba* and *Tabernaemontana divaricata* mixed with oil of *Semicarpus anacardium* and applied as a poultice in case of udder infection, whereas 53.75% of the same respondents were aware of the MVD.

Observation revealed that some farmers were aware about ITKs according to the seasonal availability of a particular plant or according to their convenience. Some farmers were only aware but were not using it properly. Some farmers were aware about particular ITKs but lack any knowledge about form and doses of application. About Modern Veterinary Drug (MVD), some respondents told that they used it "as prescribed by the veterinary doctor", whereas, some were aware about the name of a particular drug used for a particular disease. Some farmers told that veterinarian had given him some drugs in tablet forms or in liquid form but he was not aware about the name of that particular drug.

4.5 Sources and channels of filtration and transfer or dissemination of knowledge among ITK practitioners and livestock owner

4.5.1 Traditional healers

4.5.1.1 Source of awareness for traditional healers (N=30) in the selected villages of West Singhbum district, Jharkhand

Among 30 local healers interviewed, it was found that majority of the healers (56.66%) were aware about the several indigenous practices which were gathered from their ancestors and rest of the respondents (43.34%) had got the information for the first time from their gurus (Table 8).

4.5.1.2 Transfer of practice from traditional healers to others (N=30) in the selected villages of West Singhbhum district, Jharkhand

Among 30 local healers it was found that majority of the local healers (46.67%) disseminate their knowledge to their son followed by 26.67% to very reliable disciples and 13.34% to their relatives. 13.34% local healers were found not willing to transfer their knowledge of practice to anybody (Table 9).

It was also observed that the healers were reluctant to reveal their knowledge about ITKs. However after establishing a rapport with them, they agreed to share. Most of them disseminate their knowledge to their family members especially son or close relatives. Some were found to transfer the knowledge to very reliable disciples who had to accompany them for a long time and to gain their confidence.

4.5.2 From Livestock farmers

4.5.2.1 Source of awareness among traditional farmers (N=320) in the selected villages of West Singhbhum district, Jharkhand

Among 320 traditional farmers, it was found that 35.63% of the respondents were aware for the first time about the ITKs from family members, followed by 25.94% from friend, 14.07% from neighbors, 12.82% from relative and 11.57% from direct observation (Table 10).

Table 8: Source of awareness for traditional healers (N=30) in the selected villages of West Singhbhum district, Jharkhand

Source of awareness	No. of traditional healers	Percentage
Guru	13	43.34
Ancestors	17	56.66

Table 9: Transfer of practice from traditional healers to others (N=30) in the selected villages of West Singhbhum district, Jharkhand

To whom convey	No. of traditional healers	Percentage
Very reliable disciple	8	26.67
Son	14	46.67
Relative	4	13.34
None	4	13.34

Table 10: Source of awareness among traditional farmers (N=320) in the selected villages of West Singhbhum district, Jharkhand

Source of awareness	No. of livestock owners	Percentage
Family member	114	35.63
Friend	83	25.94
Neighbors	45	14.07
Relative	41	12.82
Direct observation	37	11.57

Table 11: Transfer of practice from traditional farmers to others (N=320) in the selected villages of West Singhbhum district, Jharkhand

Source of awareness	No. of livestock owners	Percentage
Family members, Friends, Neighbors, relatives	49	15.32
Family members, Friends	186	58.13
Family members	85	26.57

4.5.2.2 Transfer of practice from traditional farmers to others (N=320) in the selected villages of West Singhbhum district, Jharkhand

Among 320 traditional farmers, it was found that 58.13% of the respondents disseminate their knowledge on ITK to family members, friends whereas, 26.57% to family members only and 15.32% to family members, friends, neighbors and relatives (Table 11).

4.6 Validation of selected ITKs

4.6.1 Field validation

4.6.1.1 Field validation of different alternatives (MVD; ITK1-10) used for bovine mastitis by Quantification of indigenous Knowledge (QuIK) method.

For effectiveness (How many animals cured?), ANOVA of data (Table 12) revealed that there was highly significant difference between ITKs and MVD ($p<0.05$). MVD was most effective (8.60±0.54) followed by ITK 5 (7.60±1.54), ITK- 2 (7.60±0.89), ITK- 3 (7.40±0.89), ITK-6 and ITK 8 (7.20±0.44), ITK- 4 (7.00±0.70), ITK- 7 and ITK- 9 (6.60±0.54) and ITK-10 (6.20±0.44) in this regard.

Uses of all the ITKs were cost effective over MVD (Table 12). MVD was found to be costlier, whereas there was no significant difference among ITKs ($p<0.05$) in this regard.

The MVD was found to be most effective and significantly different over other alternatives with respect to quickness in healing ($p<0.05$) (Table 12), whereas, there was no significant difference between ITKs ($p<0.05$) in this regard.

About easy in preparation MVD (Table 14), was significantly different ($p<0.05$) than ITK whereas there was no significant difference among the ITKs in this regard.

Regarding side effect it was found that MVD was having more side effect than the ITKs, as perceived by the farmer. MVD was found significantly different over other ITKs ($p<0.05$) (Table 12), as far as side effect was concerned, whereas as there was no significant difference among ITKs.

As far as availability is concerned, there was significant difference between MVD and ITKs ($p<0.05$) (Table 12), whereas as there was no significant difference among ITKs.

To buy MVD farmers would have to consult veterinary doctor and purchase it from the concerned medical shop, while the ITKs can be prepared locally.

The critical perusal table 12 showed that ITKs were favorably accepted by the traditional farmers as far as cost effectiveness, side effect and availability were concerned. Besides, MVD was more effective in terms of effectiveness (How many animals cured?), quickness in healing and easy in preparation. Experience gained from discussion with the traditional farmers showed that cheaper, locally available indigenous technology with least side effects seemed to be the viable option in the study area.

4.6.1.2 Field validation by Fidelity level (FL %)

For the use of Ethnoveterinary medicinal herbs for the treatment of bovine mastitis, 320 people from respective villages were interviewed. As per the interview schedule the analysis revealed that 40 different species of plants were in use. The plant species belongs to the family Fabaceae was most frequently used herb and of which, 5 species were used by most of the respondent. Other plant used for treatment belonged to family Apiaceae, Zingiberaceae, Piperaceae, Rutaceae and Solanaceae. Information on 40 plant species belonged to 29 families (Table 13, Fig. 2 to 41) was collected as disclosed by the respondents in the study. The Fidelity value (FL) of plant species (Table 13) for a specific disease in the present study area varied between 20 and 95%. Medicinal plants having more than 70% FL values are *Listea monopetala* (*L. monopetala*) (95%) followed by *Piper longum* (*P. longum*) (85%), *Glycyrrhiza glabra* (*G.glabra*) (80%), *Terminalia bellerica* (*T. bellerica*) *(80%), Bombax ceiba (B. ceiba) (75%), Butea monosperma (B. monosperma)* (75%).

4.6.1.3 Field validation of 6 different plant species (*Listea monopetala*, *Glycyrrhiza glabra*, *Terminalia bellerica, Bombax ceiba, Piper longum* and *Butea monosperma*) used against bovine mastitis by QuIK method

For the validation of traditional indigenous veterinary practices (TIVP), Matrix of decision criteria with respect to the practices for curing of bovine mastitis presented in table 14 was prepared and administered to 20 key informants individually. Matrix ranking for the relative performance of the options with respect to each criteria viz. quickness in recovery, availability, easy in preparation and low level of side effect was done with scoring pattern of 1 to 10. The

Table 12: Evaluation on respondents to different alternatives (MVD; ITK1-10) used for bovine mastitis (N=20) (Mean±SD) for the selected villages of West Singhbhum district, Jharkhand

Criteria/ Alternatives	Effectiveness	Cost Effectiveness	Quickness in healing	Easy in preparation	Side effect	Availability
MVD	8.60±0.54^{B}	4.60±0.54^{A}	8.40±0.54^{B}	9.20±0.44^{B}	7.20±0.44^{A}	6.60±0.54^{A}
ITK- 1	7.20±0.44^{AB}	9.20±0.44^{B}	5.20±0.44^{A}	5.60±0.89^{A}	9.20±0.44^{B}	9.20±0.44^{B}
ITK- 2	7.60±0.89^{AB}	9.40±0.54^{B}	5.40±0.54^{A}	5.80±0.83^{A}	9.20±0.44^{B}	9.40±1.34^{B}
ITK- 3	7.40±0.89^{AB}	9.20±0.44^{B}	6.20±0.44^{A}	5.80±0.44^{A}	9.00±1.00^{B}	9.60±0.89^{B}
ITK- 4	7.00±0.70^{A}	9.60±0.89^{B}	6.20±0.44^{A}	6.40±0.54^{A}	9.40±0.54^{B}	9.60±0.54^{B}
ITK- 5	7.60±1.54^{AB}	9.20±1.09^{B}	5.80±0.83^{A}	5.60±0.54^{A}	9.40±0.54^{B}	9.20±0.44^{B}
ITK- 6	7.20±0.44^{AB}	9.40±0.54^{B}	6.20±0.44^{A}	5.80±0.83^{A}	9.40±0.89^{B}	9.40±0.54^{B}
ITK- 7	6.60±0.54^{A}	9.80±0.44^{B}	5.20±0.44^{A}	5.20±1.09^{A}	9.60±0.54^{B}	8.40±0.89^{B}
ITK- 8	7.20±0.44^{AB}	9.60±0.54^{B}	5.60±0.54^{A}	5.20±0.83^{A}	9.00±1.00^{B}	8.80±1.09^{B}
ITK- 9	6.60±0.54^{A}	9.60±0.54^{B}	5.20±0.44^{A}	5.40±1.51^{A}	9.20±0.83^{B}	9.20±0.44^{B}
ITK- 10	6.20±0.44^{A}	9.80±0.44^{B}	5.20±0.44^{A}	5.20±0.44^{A}	9.00±0.70^{B}	9.20±0.44^{B}

*Values with different superscripts are significant at level ($p<0.05$) in rows (A, B)

Table 13: Use of medicinal plants for the treatment and control of bovine mastitis in district West Singhbhum, Jharkhand

Sl. No.	Vernacular name	Botanical name	Family	Part used	Mode of preparation and application	Fidelity Level (FL)
1	Agnijhal	*Clausena excavata* Burm.f.	Rutaceae	Root	Root is ground into paste and used as a poultice on udder till healing	35%
2	Amaltas	*Cassia fistula* L.	Caesalpinaceae	Root	Root powder is applied on udder to reduce inflammation, hemorrhages and wound on udder	30%
3	Amla	*Phyllanthus emblica* L.	Euphorbiaceae	Leaf and fruit	The leaf and fruit paste helps to prevent infection and healing of ulcers when applied topically on udder	40%
4	Anar	*Punica granatum* L.	Punicace	Leaf	Leaves are ground into paste and applied on udder to reduce infection	55%
5	Aswagandha	*Withania somnifera* L.	Solanaceae	Roots	Fresh root paste applied topically on udder	20%
6	Bael	*Aegle marmelos* L.	Rutaceae	Leaves	Leaves paste applied on udder as hot fomentation	35%
7	Bahera	*Terminalia belerica* Roxb	Combretaceae	Fruit	*Terminalia belerica* along with roots of *Asparagus racemosa*, seeds of fenugreek and fennel, black pepper, mustard oil, onion crushed together boil in water upto one fourth of original volume and to be given orally	80%
8	Bhilwa	*Semicarpus anacardium* Linn.	Anacardiaceae	Seeds	Oil obtained from seed applied as antiseptic on udder wound	55%
9	Biranga	*Embelia ribes* Burm F.	Myrsinaceae	Seeds	Seed mixed with fruit of *Phyllanthus emblica* then make powder and drenched orally	40%
10	Brahmi/Beng sag	*Centella asiatica* L.	Apiaceae	Leaves	Leaves paste is applied as a poultice in case of udder infection	60%
11	Chandni/ Crape jasmine	*Tabernaemontana divaricata* L.	Apocynaceae	Leaf	The leaves are softened by fire and applied to relieve inflammation of udder	40%
12	Curry leaves	*Murraya koenigii* L.	Rutaceae	Leaves	Leaves paste applied on udder to prevent infection	30%
13	Dhatura	*Datura alba* L.	Solanaceae	Leaves and fruit	Leaves paste can applied over hemorrhoids in case of skin ulcers of udder and fruit is roasted and ground to make a fine powder, sesame oil (250g) is added to make a paste and to be applied on the infected part of udder	65%
14	Elaichi	*Elettaria cardamomum* L.	Zingiberaceae	Seeds	Elaichi seeds along with seeds of fenugreek and fennel, black pepper, mustard oil, dry ginger powder helps in reducing fever in case of udder infection	35%

Table 13: Contd...

Sl. No.	Vernacular name	Botanical name	Family	Part used	Mode of preparation and application	Fidelity Level (FL)
15	Golki	*Piper nigrum* L.	Piperaceae	Fruit	Powder of fruit mixed with ghee and drenched orally in case of mastitis	45%
16	Guduchi	*Tinospora cordifolia*	Menispermaceae	Stem	Stem paste applied on the udder to reduce inflammation and infection	65%
17	Haldi	*Curcuma longa* L.	Zingiberaceae	Rhizome	Warm paste of *Curcuma longa* (powder) is mixed with leaf of datura plant and applied externally to the mammary glands to cure mastitis	70%
18	Hing	*Ferula asafoetida* Linn.	Apiaceae	Gum gathered from the roots	Small quantity of hing dissolved in water applied on teats	40%
19	Jatamansi	*Nardostachys Jatamansi*	Valerianaceae	Rhizome	The herb is mixed with cold water to form a paste and reduce burning sensation, inflammation, pain and improving skin texture of udder	55%
20	Kala jira	*Nigella sativa* L.	Ranunculaceae	Seed	The seed paste along with honey to be applied on bovine udder to prevent mastitis	35%
21	Kaphal	*Myrica nagi* Linn.	Myricaceae	Bark	Bark paste applied on infected part of cow's udder to heal mastitis	40%
22	Kantamari sag	*Amaranthus spinosus* L.	Amaranthaceae	Roots	Warm paste of root paste applied on whole udder to reduce inflammation	50%
23	Karanj	*Pongamia pinnata* L.	Fabaceae	Seed	Seed oil applied on inflamed part of udder to prevent infection	55%
24	Kuth	*Saussurea costus*	Asteraceae	Roots	Infusion used as lotion in chronic mastitis and helps to arrest bleeding	45%
25	Kutki	*Picrorhiza kurroa*	Plantaginaceae	Root	The root is administered orally along with jaggery	40%
26	Mehandi	*Lawsonia inermis* L.	Lythraceae	Leaf, Bark	Paste of leaves and bark applied on udder to reduce pain, swelling and infection.	45%
27	Mulethi	*Glycyrrhiza glabra* L.	Fabaceae	Roots	Root paste of *Glycyrrhiza glabra* and *Cucurma longa* applied on udder to reduce infection	80%
28	Neem	*Azadirachta indica* L.	Meliaceae	Leaf, Bark	Leaves and bark paste can applied on udder to decrease the microbial load	70%

Table 13: Contd...

Sl. No.	Vernacular name	Botanical name	Family	Part used	Mode of preparation and application	Fidelity Level (FL)
29	Nenua/ Sponge gouard	*Luffa cylindrica*	Cucurbitaceae	Leaves	Leaves paste applied on udder in case of gangrenous mastitis	65%
30	Palash	*Butea monosperma* Lamk.	Fabaceae	Bark and flowers	Juice extracted from bark and flower is applied on udder to reduce infection	75%
31	Patal kumra	*Pereira tuberose*	Fabaceae	Tuber	Tuber and hing paste used in boils, ulcer and wounds on udder	65%
32	Pipali	*Piper longum* L.	Piperaceae	Fruits	Decoction of fruits administered orally to reduce fever in case of mastitis	85%
33	Pojo	*Listea monopetala*	Lauraceae	Bark	Bark paste applied on udder to cure abscesses and to reduce swelling	95%
34	Lehsun	*Allium sativum* L.	Alliaceae	Bulbs	250g grinded with ghee and administered orally for 7 days	65%
35	Sanai patta	*Crotalaria verrucosa* L.	Fabaceae	Leaves	Juice of leaves applied on udder to combat infection	40%
36	Sarsson	*Brassica Compestris* L.	Brassicaceae	Leaf, seed	Leaves used as poultice over abscess on udder, seed oil 500ml administered orally for 7 days	60%
37	Satavari	*Asparagus racemosa*	Asparagaceae	Fleshy roots	Roots grinded into paste and drenched orally for 7 days	65%
38	Saunf	*Foeniculum Vulgare* Mill.	Apiaceae	Seed	Seeds roasted and mixed in vegetable oil and administered orally for 4 days	55%
39	Semal	*Bombax ceiba* L.	Malvaceae	Fresh bark	Bark grind into paste mixed with little water and applied on the infected part of udder and decoction of the bark is also given orally to combat infection	75%
40	Tulsi	*Oscimum sanctum* L.	Lamiaceae	Leaf	Leaves grounded into paste and applied on udder	70%

Table 14: Validation of 6 most useful herbal plant materials for traditional ethno veterinary practices for curing bovine mastitis by the tribal people of West Singhbhum district of Jharkhand, India (N=20) (Mean ± SD)

Criteria/ Alternatives	Effectiveness	Cost Effectiveness	Quickness in healing	Easy in preparation	Side effect	Availability
Mulethi (Glycyrrhiza glabra)	5.90±0.73 ^{ABC}	9.30±0.48 ^{A}	4.80±0.42 ^{AB}	5.60±0.51 ^{B}	8.50±0.52 ^{A}	7.50±0.52 ^{A}
Pojo (Listea monopetala)	6.70±0.67 ^{C}	9.00±0.66 ^{A}	5.00±0.47 ^{B}	4.30±0.31 ^{AB}	8.90±0.73 ^{A}	7.90±0.73 ^{A}
Pipali (Piper longum)	4.90±0.73 ^{A}	9.20±0.63 ^{A}	4.40±0.84 ^{A}	5.10±0.73 ^{AB}	8.80±0.63 ^{A}	7.80±0.78 ^{A}
Palash (Butea monosperma)	6.40±0.69 ^{BC}	9.20±0.63 ^{A}	4.10±0.56 ^{AB}	5.20±0.78 ^{AB}	9.00±0.66 ^{A}	7.90±0.73 ^{A}
Bahera (Terminalia belerica)	5.50±0.70 ^{AB}	9.40±0.69 ^{A}	4.70±0.82 ^{AB}	5.10±0.56 ^{AB}	8.40±0.69 ^{A}	8.00±0.94 ^{A}
Semal (Bombex ceiba)	5.80±1.03 ^{ABC}	9.30±0.48 ^{A}	4.53±0.70 ^{A}	4.60±0.51 ^{A}	8.80±0.63 ^{A}	7.80±0.63 ^{A}

*Values with different superscripts are significant at level ($p<0.05$) in columns (A, B, C)

Table 15: Isolation of major mastitis causing pathogens from individual lactating cows suffering from subclinical and clinical mastitis

Sl No.	Cow no.	Type of mastitis	Selective media/ Test	Organisms Identified
1	2 RF	SCM	Edward's media/ Hoti's Test	*St. agalalactiae*
2	28 LH	SCM	Mannitol Salt Agar	*S. aureus*
3	52 LH	SCM	Mannitol Salt Agar/ Coagulase Test	CNS
4	41 RF	SCM	EMB/ Mac Conkey Agar	*E. Coli*
5	40 RF	SCM	Mannitol Salt Agar/ Coagulase Test	CNS
6	32 LH	SCM	Mannitol Salt Agar/ Coagulase Test	CNS
7	44 LF	SCM	EMB/ Mac Conkey Agar	*E. Coli*
8	17 RF	SCM	Edward's media media/ Hoti's Test	*St. agalalactiae*
9	15 LF	SCM	Mannitol Salt Agar	*S. aureus*
10	8 LH	SCM	Edward's media media/ Hoti's Test	*St. agalalactiae*
11	57 LF	SCM	Mannitol Salt Agar	*S. aureus*
12	37 RF	SCM	Edward's media media/ Hoti's Test	*St. agalalactiae*
13	56 RH	SCM	Edward's media media/ Hoti's Test	*St. agalalactiae*
14	30 RH	SCM	Mannitol Salt Agar	*S. aureus*
15	48 RF	SCM	Edward's media media/ Hoti's Test	*St. agalalactiae*
16	50 LH	SCM	Edward's media media/ Hoti's Test	*St. agalalactiae*
17	42 RF	CM	Mannitol Salt Agar	*S. aureus*
18	14 LF	SCM	EMB/ Mac Conkey Agar	*E. Coli*
19	5 RH	SCM	Mannitol Salt Agar/ Coagulase Test	CNS
20	11 LH	CM	Mannitol Salt Agar	*S. aureus*

Fig. 2: Root of *Clausena excavate*

Fig. 3: Root of *Cassia fistula*

Fig. 4: Fruit of *Embllica officinalis*

Fig. 5: Fruit and leaf of *Punica granatum*

Fig. 6: Roots of *Withania somnifera*

Fig. 7: Leaves of *Aegle marmelos*

Fig. 8: Fruits of *Terminalia bellerica*

Fig. 9: Seeds of *Semecarpus anacardium*

Fig. 10: Seeds of *Embelia ribes*

Fig. 11: Leaves of *Centenella asiatica*

Fig. 12: Leaves of *Tabernaetoma divaricata*

Fig. 13: Leaves of *Murraya Koenigii*

Fig. 14: Leaves and fruits of *Datura alba*

Fig. 15: Seeds of *Elettaria cardamomum*

Fig. 16: Fruit of *Piper nigrum*

Fig. 17: Stem of *Tinospora cordifolia*

Fig. 18: Rhizome of *Cucurma longa*

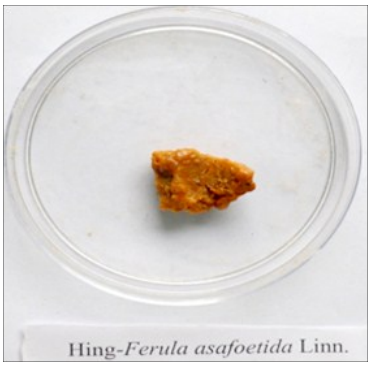


Fig. 19: Root gum of *ferula asafoetida*

Fig. 20: Rhizome of *Nordostachys jatamansi*

Fig. 21: Seeds of *Nigella sativa*

Fig. 22: Bark of *Myrica nagi*

Fig. 23: Roots of *Amaranthus spinosus*

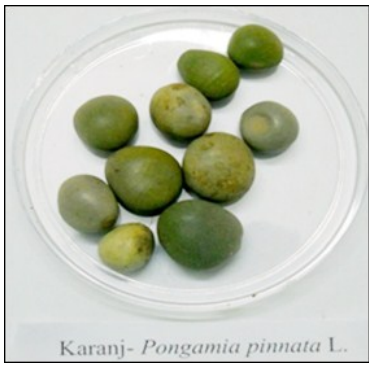


Fig. 24: Seeds of *Pongamia pinnata*

Fig. 25: Roots of *Saussurea costus*

Fig. 26: Roots of *Picrorhiza kurroa*

Fig. 27: Leaves of *Lawsonia inermis*

Fig. 28: Roots of *Glycyrrhiza glabra*

Fig. 29: Leaves of *Azadirachta indica*

Fig.30: Leaves of *Luffa cylindrica*

Fig. 31: Bark of *Butea monosperma*

Fig. 32: Tuber of *Pereira tuberose*

Fig. 33: Fruit of *Piper longum*

Fig. 34: Bark of *Listea monopetala*

Fig. 35: Bulb of *Allium sativum*

Fig. 36: Leaves of *Crotalaria verrucosa*

Fig. 37: Seeds of *Brassica compestris*

Fig. 38: Roots of *Asparagus racemosa*

Fig. 39: Seeds of *Foeniculum vulgare*

Fig. 40: Bark of *Bombax ceiba*

Fig. 41: Leaves of *Oscimum sanctum*

result showed the differences in TIVP. *Listea monopetala* (6.70±0.67) ranked higher in effectiveness and quickness in healing than *Listea monopetala*, *Glycyrrhiza glabra, Terminalia bellerica, Bombex ceiba, Piper longum* and *Butea monosperma* ($p<0.05$). There was no significant difference in cost effectiveness, side effect and availability of *Listea monopetala*, *Glycyrrhiza glabra, Terminalia bellerica, Bombax ceiba, Piper longum* and *Butea monosperma.* Regarding easy in preparation *Glycyrrhiza glabra* ranked higher than other plant species (Table 14).

4.6.2 Laboratory validation of 6 different plant species (*Listea monopetala Glycyrrhiza glabra. Terminalia bellerica, Bombex ceiba, Piper longum* and *Butea monosperma*) used against bovine mastitis

4.6.2.1 Collection of milk samples and isolation of major mastitis causing pathogens

Cows affected with sub clinical mastitis (SCM) were screened by California mastitis test (CMT). The cows affected with clinical mastitis (CM) were screened on the basis of the clinical sign exhibited. From a total of 150 cows screened, 20 (13.33%) animals affected with mastitis were taken for sampling, out of which 18 (12%) were positive for SCM and 2 (1.33%) were positive for CM, as revealed by CMT and clinical examination respectively. Out of 20 mastitis case 6 (30%) were suffering from coagulase positive *Staphylococcus aureus*, 4 (20%) coagulase negative *Staphylococcus aureus*, 7 (35%) *Sterptococcus agalactiae* and 3(15%) *E. coli* infections are depicted in Table 15. Primary isolation of milk sample was done on 5% bovine blood agar (Fig. 42) and the causative organism was initially identified by colony morphology and odour. Then causative organism were grown on selective media like Mannitol salt agar (MSA) (Fig. 43), Baird- Parker agar (Fig. 44), 5% bovine blood agar (Fig. 45), Edward media (Fig. 46) and Eosine methylene blue (EMB) agar (Fig.47) for Coagulase positive *Staphylococcus aureus,* Coagulase negative *Staphylococcus aureus, Streptococcus agalactiae* and *E. coli*

4.6.2.2 Biochemical characterization of organisms

Pathogens identified by HiStaph™, HiStrep™ and Hi *E. coli*™ identification kit. For identification of *Staphylococcus aureus*, *Streptococcus agalactiae* and *E.coli* species, results were confirmed positive by twelve conventional biochemical tests for each kit and results were shown in (Table 16, 17, 18 and Fig. 48, 49, 50, 51, 52, 53) before and after inoculation.

4.6.2.3 Coagulase test

The coagulase test was used to differentiate between Coagulase Positive *Staphylococcus aureus* from Coagulase Negative *Staphylococcus aureus* (Fig. 54). The plasma was coagulated in case of 'positive' case and in case of 'negative' result the plasma was in liquid state.

4.6.2.4 Antibiotic sensitivity test

4.6.2.4.1 Resistance pattern against antibiotics

Antibiotic sensitivity of all the four organisms i.e. Coagulase Negative *Staphylococcus aureus,* Coagulase Positive *Staphylococcus aureus, Streptococcus agalactiae* and *E. coli* were tested against different antibiotic discs and the inhibition zones were measured in mm. The result of this study indicated that Gentamicin had the highest sensitivity to the Coagulase Negative *Staphylococcus aureus* and Coagulase Positive *Staphylococcus aureus.* Ciprofloxacin showed highest sensitivity to the *Streptococcus agalactiae* isolates. In case of *E. coli* isolates Chloramphenicol was found to be the most effective (Table 19 and Fig. 55).

4.6.2.4.2 Resistance pattern of herbal extracts at different concentrations against pathogenic organisms

The aqueous, methanolic and hydromethanolic extracts of 6 different plant species (*Listea monopetala Glycyrrhiza glabra, Terminalia bellerica, Bombex ceiba, Piper longum* and *Butea monosperma*) with the concentration of 2 mg, 4 mg, 6 mg and 8 mg showed broad spectrum of antibacterial activity against Coagulase Negative *Staphylococcus aureus* and Coagulase Positive *Staphylococcus aureus, Streptococcus agalactiae* and *E. coli* (Table 19, 20, 21, 22, 23, 24, 25).

Sensitivity pattern of methanolic extract of *Glycyrrhiza glabra, Listea monopetala and Piper longum* revealed maximum zone of inhibition against the *St. agalactiae. Glycyrrhiza glabra* showing the maximum zone of inhibition at the concentration of 2 mg (17 mm), 4 mg (18 mm), 6 mg (20 mm) and 8 mg (22 mm) (Fig. 56). For *Piper longum* showing the maximum zone of inhibition at the concentration of 2 mg (18 mm), 4 mg (19 mm), 6 mg (19 mm) and 8 mg (20 mm) (Fig. 57) and *Listea monopetala* zone of inhibition was in the concentration of

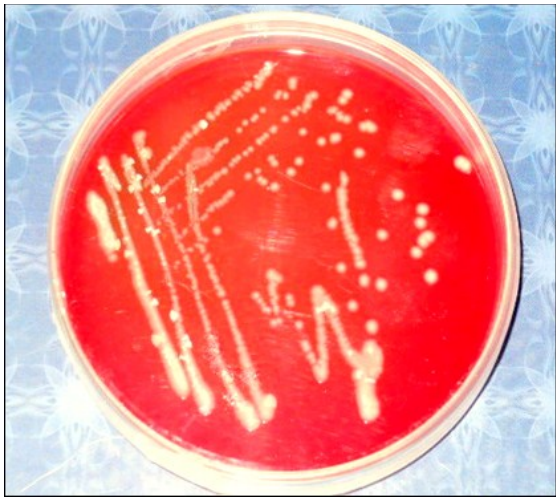


Fig. 42: Primary isolation of bacteria from mastitis milk on Blood agar plate

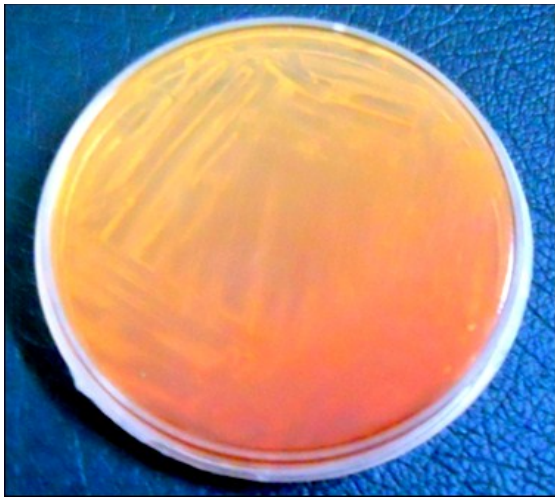


Fig. 43: Growth of *S. aureus* on Mannitol salt agar

Fig. 44: Growth of S. aureus on Baird Parker agar

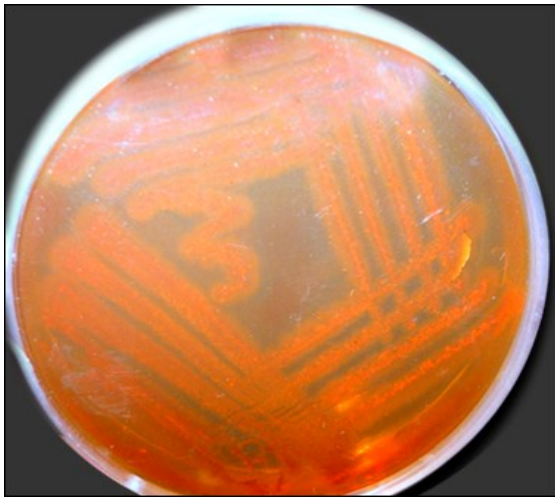


Fig. 45: Growth of *St. agalactiae* on blood agar

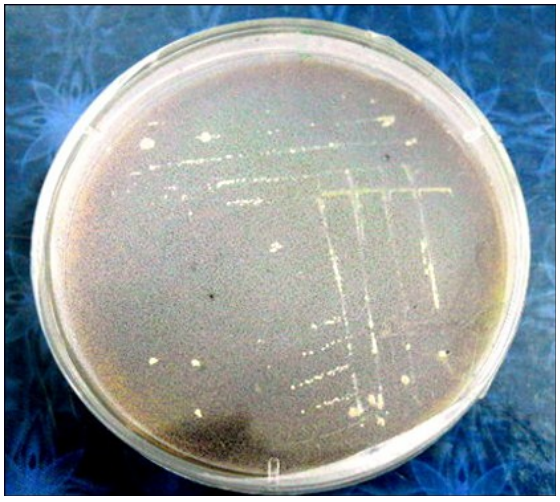


Fig. 46: Growth of *St. agalactiae* on Edward media

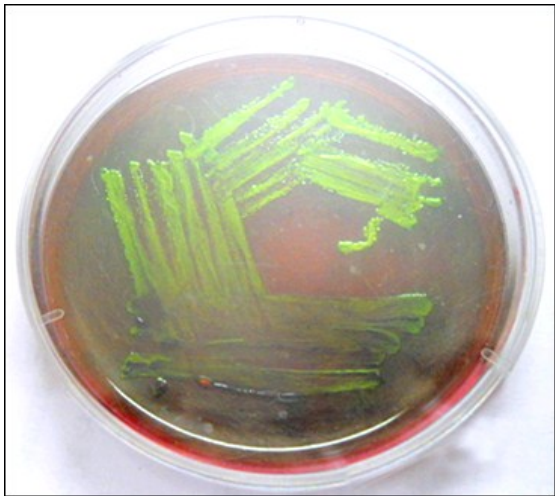


Fig. 47: Growth of *E. coli* on EMB agar

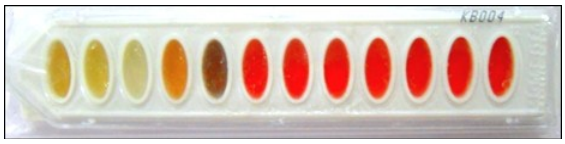


Fig. 48: Before inoculation

Fig. 49: After inoculation

Identification of *S. aureus* though Hi Staph™ identification kit

Fig. 50: Before inoculation

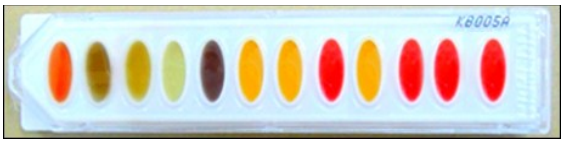


Fig. 51: After inoculation

Identification of *St. agalactiae* though Hi Strep™ identification kit

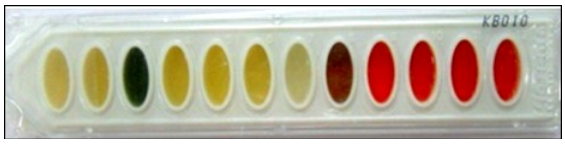


Fig. 52: Before inoculation

Fig. 53: After inoculation

Identification of *E. coli* though Hi *E. coli*™ identification kit

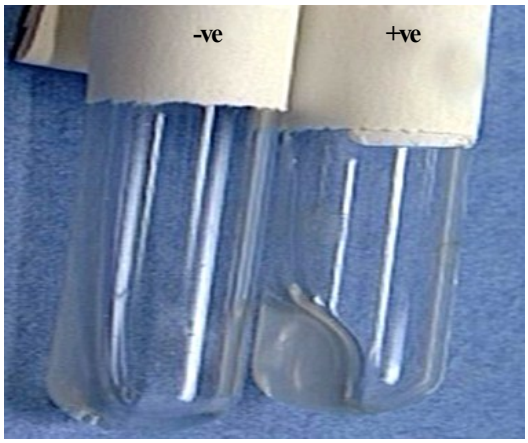


Fig. 54: Coagulase test

Table 16: Identification index of *Staphylococcus aureus* Species

Organisms	Voges Proskaeur's	Alkaline Phosphatase	ONPG	Urease	Arginine utilization	Mannitol	Sucrose	Lactose	Arabinose	Raffinose	Trehalose	Maltose
Staphylococcus aureus	+	+	-	+w	+w	+	+	+	-	-	+	+

+ = Positive
- = Negative
+w = Positive to weak reaction

Table 17: Identification index of *Streptococcus agalctiae* Species

Organisms	Voges Proskaeur's	Esculin hydrolysis	PYRO	NPG (β-galacto-sidase)	Arginine utilization	Glucose	Lactose	Arabinose	Sucrose	Sorbitol	Mannitol	Raffinose
Streptococcus agalactiae	+	-	-	-	+	+	d	-	+	-	-	-

+ = Positive
- = Negative
d = Detected

Table 18: Identification index of *E. coli Species*

Organisms	Methyl Red	Voges Proskaeur's	Citrate utilization	Indole	Glucoronidase	Nitrate	ONPG	Lysine	Lactose	Glucose	Sucrose	Sorbitol
E. coli	+	-	-	+	+	+	+	+	+	+	V	+

+ = Positive
- = Negative
V = Variable

Table 19: Zone of inhibition (in mm) of bacterial species per antibiotic

Sl. No.	Antibiotics (µg/disc)	Zone of Inhibition (mm)			
		Coagulase Negative Staphylococcus aureus	*Coagulase Positive Staphylococcus aureus*	*Streptococcus agalactiae*	*E. coli*
1	Cefotaxime (30µg)	25 mm	23 mm	15 mm	16 mm
2	Amoxycillin (5µg)	20 mm	19 mm	17 mm	17 mm
3	Ampicillin (10 µg)	22 mm	20 mm	16 mm	16 mm
4	Penicillin G (10 IU)	19 mm	19 mm	15 mm	13 mm
5	Enrofloxacin (5µg)	22 mm	19 mm	19 mm	12 mm
6	Erythromycin (15µg)	23 mm	22 mm	20 mm	13 mm
7	Ciprofloxacin (5µg)	25 mm	22 mm	26 mm	21 mm
8	Gentamicin (10µg)	28 mm	24 mm	21 mm	20 mm
9	Streptomycin (10µg)	12 mm	11 mm	10 mm	19 mm
10	Cotrimazole (25µg)	15 mm	14 mm	20 mm	26 mm
11	Chloramphenicol (30 µg)	15 mm	14 mm	23 mm	28 mm

Table 20: Antimicrobial activity of *Glycyrrhiza glabra* showing zone of inhibition (in mm) against microorganisms

Sl. No.	Solvent	Zone of Inhibition (mm)				
		Concentration of extracts	*Coagulase Negative Staphylococcus aureus*	*Coagulase Positive Staphylococcus aureus*	*Streptococcus agalactiae*	*E. coli*
1	Aqueous	2 mg	10 mm	9 mm	12 mm	Negative
		4 mg	12 mm	10 mm	14 mm	Negative
		6 mg	14 mm	11 mm	16 mm	Negative
		8 mg	17 mm	12 mm	18 mm	Negative
2	Methanolic	2 mg	15 mm	13 mm	17 mm	13 mm
		4 mg	17 mm	14 mm	18 mm	14 mm
		6 mg	18 mm	15 mm	20 mm	16 mm
		8 mg	20 mm	16 mm	22 mm	17 mm
3	Hydromethanolic	2 mg	12 mm	10 mm	14 mm	12 mm
		4 mg	13 mm	11 mm	15 mm	13 mm
		6 mg	14 mm	12 mm	16 mm	14 mm
		8 mg	15 mm	13 mm	17 mm	15 mm
4	Ciprofloxacin	5µg	25 mm	22 mm	26 mm	21 mm

Table 21: Antimicrobial activity of *Piper longum* showing zone of inhibition (in mm) against microorganisms

Sl. No.	Solvent	Zone of Inhibition (mm)				
		Concentration of extracts	*Coagulase Negative Staphylococcus aureus*	*Coagulase Positive Staphylococcus aureus*	*Streptococcus agalactiae*	*E. coli*
1	Aqueous	2 mg	13 mm	12 mm	10 mm	8 mm
		4 mg	15 mm	13 mm	11 mm	9 mm
		6 mg	16 mm	13 mm	12 mm	10 mm
		8 mg	18 mm	14 mm	13 mm	11 mm
2	Methanolic	2 mg	14 mm	12 mm	18 mm	13 mm
		4 mg	15 mm	13 mm	19 mm	14 mm
		6 mg	16 mm	14 mm	19 mm	15 mm
		8 mg	17 mm	15 mm	20 mm	16 mm
3	Hydromethanolic	2 mg	12 mm	10 mm	13 mm	10 mm
		4 mg	13 mm	11 mm	14 mm	11 mm
		6 mg	14 mm	13 mm	15 mm	12 mm
		8 mg	16 mm	14 mm	16 mm	13 mm
4	Ciprofloxacin	5 µg	25 mm	22 mm	26 mm	21 mm

Table 22: Antimicrobial activity of *Listea monopetala* showing zone of inhibition (in mm) against microorganisms

Sl. No.	Solvent	Zone of Inhibition (mm)				
		Concentration of extracts	*Coagulase Negative Staphylococcus aureus*	*Coagulase Positive Staphylococcus aureus*	*Streptococcus agalactiae*	*E. coli*
1	Aqueous	2 mg	Negative	Negative	Negative	Negative
		4 mg	Negative	Negative	Negative	Negative
		6 mg	Negative	Negative	Negative	Negative
		8 mg	Negative	Negative	Negative	Negative
2	Methanolic	2 mg	12 mm	11 mm	18 mm	Negative
		4 mg	13 mm	12 mm	19 mm	Negative
		6 mg	14 mm	13 mm	20 mm	Negative
		8 mg	15 mm	14 mm	21 mm	Negative
3	Hydromethanolic	2 mg	10 mm	9 mm	7 mm	Negative
		4 mg	11 mm	10 mm	7 mm	Negative
		6 mg	12 mm	12 mm	8 mm	Negative
		8 mg	13 mm	13 mm	8 mm	Negative
4	Ciprofloxacin	5 µg	25 mm	22 mm	26 mm	21 mm

Table 23: Antimicrobial activity of *Terminalia bellerica* showing zone of inhibition (in mm) against microorganisms

Sl. No.	Solvent	Zone of Inhibition (mm)				
		Concentration of extracts	*Coagulase Negative Staphylococcus aureus*	*Coagulase Positive Staphylococcus aureus*	*Streptococcus agalactiae*	*E. coli*
1	Aqueous	2 mg	Negative	Negative	Negative	Negative
		4 mg	Negative	Negative	Negative	Negative
		6 mg	Negative	Negative	Negative	Negative
		8 mg	Negative	Negative	Negative	Negative
2	Methanolic	2 mg	13 mm	12 mm	14 mm	8 mm
		4 mg	14 mm	13 mm	15 mm	9 mm
		6 mg	15 mm	14 mm	16 mm	10 mm
		8 mg	16 mm	15 mm	17 mm	11 mm
3	Hydromethanolic	2 mg	10 mm	8 mm	12 mm	7 mm
		4 mg	11 mm	9 mm	13 mm	8 mm
		6 mg	12 mm	10 mm	14 mm	9 mm
		8 mg	13 mm	11 mm	15 mm	10 mm
4	Ciprofloxacin	5 µg	25 mm	22 mm	26 mm	21 mm

Table 24: Antimicrobial activity of *Bombex ceiba* showing zone of inhibition (in mm) against microorganisms

Sl. No.	Solvent	Zone of Inhibition (mm)				
		Concentration of extracts	*Coagulase Negative Staphylococcus aureus*	*Coagulase Positive Staphylococcus aureus*	*Streptococcus agalactiae*	*E. coli*
1	Aqueous	2 mg	Negative	Negative	Negative	Negative
		4 mg	Negative	Negative	Negative	Negative
		6 mg	Negative	Negative	Negative	Negative
		8 mg	Negative	Negative	Negative	Negative
2	Methanolic	2 mg	Negative	Negative	Negative	Negative
		4 mg	Negative	Negative	Negative	Negative
		6 mg	Negative	Negative	Negative	Negative
		8 mg	Negative	Negative	Negative	Negative
3	Hydromethanolic	2 mg	Negative	Negative	Negative	Negative
		4 mg	Negative	Negative	Negative	Negative
		6 mg	Negative	Negative	Negative	Negative
		8 mg	Negative	Negative	Negative	Negative
4	Ciprofloxacin	5 µg	25 mm	22 mm	26 mm	21 mm

Table 25: Antimicrobial activity of *Butea monosperma* showing zone of inhibition (in mm) against microorganisms

Sl. No.	Solvent	Concentration of extracts	Zone of Inhibition (mm)			
			Coagulase Negative Staphylococcus aureus	*Coagulase Positive Staphylococcus aureus*	*Streptococcus agalactiae*	*E. coli*
1	Aqueous	2 mg	Negative	Negative	Negative	Negative
		4 mg	Negative	Negative	Negative	Negative
		6 mg	Negative	Negative	Negative	Negative
		8 mg	Negative	Negative	Negative	Negative
2	Methanolic	2 mg	Negative	Negative	Negative	Negative
		4 mg	Negative	Negative	Negative	Negative
		6 mg	Negative	Negative	Negative	Negative
		8 mg	Negative	Negative	Negative	Negative
3	Hydromethanolic	2 mg	Negative	Negative	Negative	Negative
		4 mg	Negative	Negative	Negative	Negative
		6 mg	Negative	Negative	Negative	Negative
		8 mg	Negative	Negative	Negative	Negative
4	Ciprofloxacin	5 µg	25 mm	22 mm	26 mm	21 mm

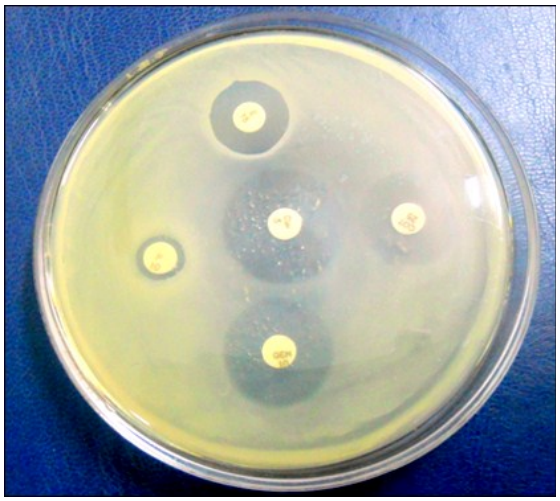


Fig. 55: Antibiotic disc sensitivity test (ABST) of different antimicrobial discs for *St. agalactiae*

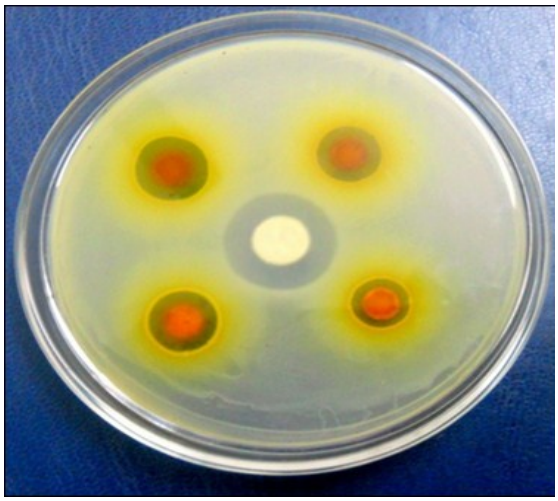


Fig. 56: ABST of methanolic extract of *Glycyrrhiza glabra* against *St. agalactiae*

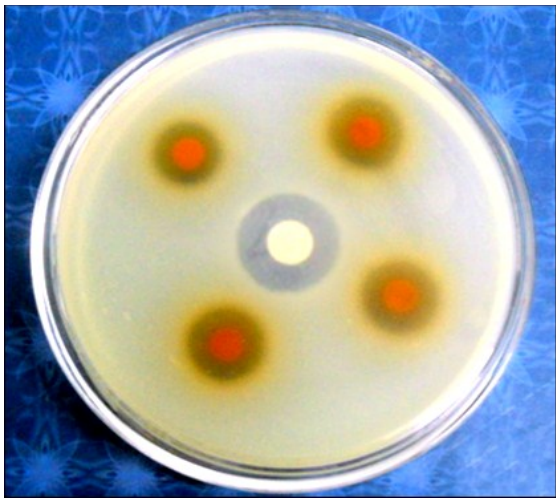


Fig. 57: ABST of methanolic extract of *Piper longum* against *St. agalactiae*

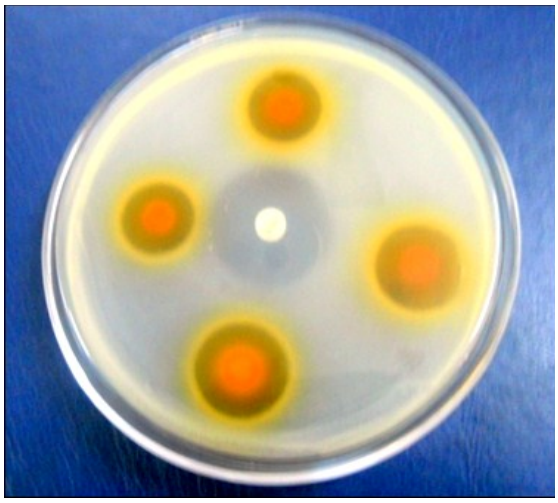


Fig. 58: ABST of methanolic extract of *Listea monopetala*

2 mg (18 mm), 4 mg (19 mm), 6 mg (20 mm) and 8 mg (21 mm) (Fig. 58). Therefore these extracts were chosen for further therapeutic trail in mice mastitis model.

Antimicrobial activity of aqueous, methanolic and hydromethanolic extracts of *Bombex ceiba* and *Butea monosperma* showed no activity against Coagulase negative *Staphylococcus aureus and* Coagulase positive *Staphylococcus aureus, Streptococcus agalactiae* and *E. coli*.

4.6.2.4.3 Determination of minimum inhibitory concentration (MIC)

All extract were subjected to broth double dilution method to determine MIC (Table 26). The maximum activity was observed against *St. agalactiae* by methanolic extract of *G. glabra* and *L. monopetala* at a concentration of 1.56 mg/ml and 6.25 mg/ml for *P. longum* respectively (Fig. 59, 60, 61). Aqueous and hydromethanolic extract of *G. glabra*, *L. monopetala* and *P. longum* against *E. coli* showed no result.

4.6.2.5 Phytochemical analysis

i. ***G. glabra*** **–** Alkaloids was absent in all the extracts aqueous, methanolic and hydromethanolic. Steroids was absent in aqueous extract but present in methanolic and hydromethanolic extracts. Glycosides, saponins, tannins and flavanoids present in all the type of extracts aqueous, methanolic and hydromethanolic (Table 27).

ii. ***L. monopetala*****-** Glycosides and saponins were absent in all the types of extracts aqueous, methanolic and hydromethanolic. Whereas, tannins, flavanoids and steroids present in all the types of extracts (Table 27).

iii. ***P. longum*** **–** Alkaloids and tannins present in aqueous extract. Whereas glycosides, saponins, steroids and flavonoids were absent in aqueous extract. Saponins was absent in methanolic extract but glycosides, alkaloids, tannins and flavanoids present in methanolic extract. In hydromethhanolic extracts saponins was absent but glycosides, alkaloids, tannins and flavonoids were present (Table 27).

4.7 Therapeutic trial in laboratory animals

4.7.1 Selection of most suitable three plant extracts on the basis of ABST and MIC

Only methanolic extracts of *G. glabra*, *L. monopetala* and *P. longum* were showing best result in ABST and MIC against *St. agalactiae* hence selected for further therapeutic trial in mice.

4.7.2 Determination of colony forming unit (cfu) of challenge strain

St. agalactiae was selected as experimental pathogenic strain for the induction of mastitis in mouse model and therapeutic trial thereafter. Determination of cfu of 24 hr incubated broth cultures of *St. agalactiae* was done by serial dilution method. The mean count of cfu/ml of 24 hr incubated broth cultures of *St. agalactiae* was estimated as 10^8 cfu/ml at 10^{-5} dilution.

4.7.3 Challenge of mammary glands

Forty two, 7 days post litter healthy mice were divided into 7 groups having 6 mice in each group. Mice were challenged with 24 hr incubated broth cultures of *St. agalactiae* at the rate of 0.1 ml containing approximately 10^8 cfu/ml to the R4 and L4 mammary glands (Fig. 62). After 24 h of challenge, the animals were observed for their general condition and gross pathological changes in the inoculated mammary glands. The remaining 6 lactating healthy mice were kept as healthy control (Group I).

4.7.4 Gross examination of mice mammary gland on the basis of clinical score card for evaluation of general clinical condition pre and post challenge (PC)

The total clinical score (TCS) of mice in various groups at different time intervals were depicted in table 28.

4.7.4.1 Pre challenge score card

All the animals in all the groups were found clinically normal as revealed by score card evaluation.

4.7.4.2 Twenty-four hours post challenge

After 24 h of challenge, almost all the infected animals were found dull and depressed. Varying degrees of swelling and reddish discoloration was observed in the inoculated mammary

Table 26: Minimum inhibitory concentration of the methanolic extract of the selected herbs

Test organisms	Methanolic extracts	MIC (mg/ml)		
		Aqueous	Methanolic	Hydromethanolic
Coagulase Positive *S. aureus*	*G. glabra*	25	12.5	25
	L. monopetala	Nil	3.12	6.25
	P. longum	25	6.25	12.5
Coagulase Negative *S. aureus*	*G. glabra*	12.5	12.5	12.5
	L. monopetala	Nil	6.25	50
	P. longum	25	25	25
St. agalactiae	*G. glabra*	3.12	1.56	12.5
	L. monopetala	Nil	1.56	25
	P. longum	25	6.25	25
E. coli	*G. glabra*	Nil	50	Nil
	L. monopetala	Nil	25	Nil
	P. longum	Nil	50	Nil

Table 27: Phytochemical parameters of aqueous, methanolic and hydromethanolic extract of *Glycyrrhiza glabra*, *Listea monopetala* and *Piper longum*

Phytochemicals	Aqueous			Methanolic			Hydromethanolic		
	G. glabra	*P. longum*	*L. monopetala*	*G. glabra*	*P. longum*	*L. monopetala*	*G. glabra*	*P. longum*	*L. monopetala*
Alkaloids	-	+	+	-	+	+	-	+	+
Glycosides	+	-	-	+	+	-	+	+	-
Saponins	+	-	-	+	-	-	+	-	-
Tannins	+	+	+	+	+	+	+	+	+
Steroids	-	-	+	+	+	+	+	+	+
Flavanoids	+	-	+	+	+	+	+	+	+

Present: (+), Absent :(-)

Fig. 59: Minimum Inhbitory Concentration (MIC) of methanolic extract of *Glycyrrhiza glabra*

Fig. 60: MIC of methanolic extract of *Piper longum*

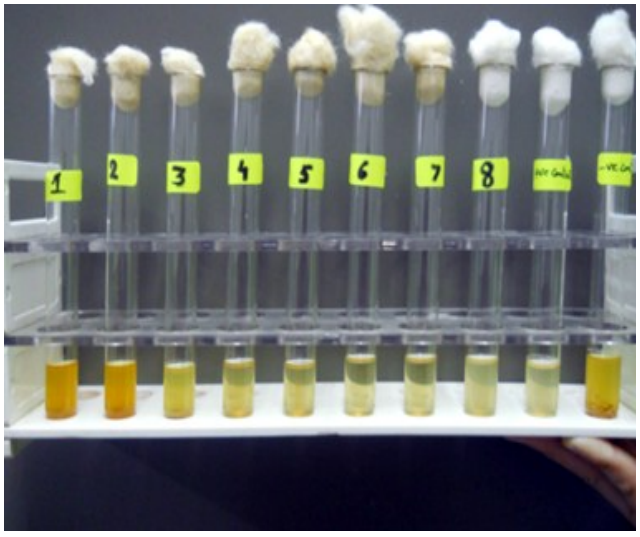


Fig. 61: MIC of methanolic extract of *Listea monopetala*

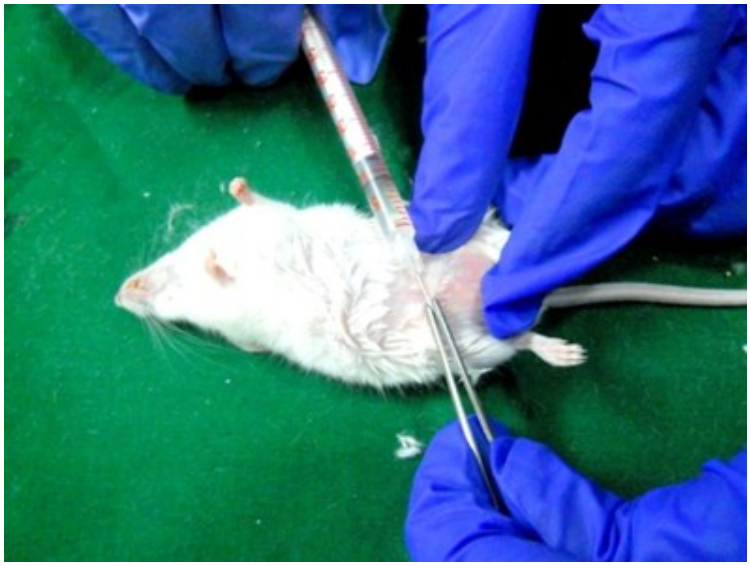


Fig. 62: Mice at the time of bacterial inoculation

glands of infected untreated control (Group II) animals at 24 h. Mice of the Group I (Negative control/Healthy) were found clinically normal with no gross pathological alterations in the mammary glands. Mice of the Group II (Positive control) were found clinically affected with slight swelling of mammary glands whereas, not much pathological changes could be observed in the challenged mammary glands of herbal extract treated animals of Group III, Group IV, Group V and Group VI and antibiotic treated Group VII animals.

4.7.4.3 Forty-eight hours post challenge

At 48 h PC, animals were observed for their general condition and no gross pathological changes in the inoculated mammary glands. Profound dullness and depression were observed in Group II animals while their mammary glands showed slight reddish discoloration. No mice of infected untreated group (Group II) were found dead. However, the challenged mammary glands of herbal extract and honey treated animals (Group III, Group IV, Group V and Group VI) and antibiotic group (Group VII) animals did not reveal much gross pathological changes, but the animals were found dull and depressed. The unchallenged Group I healthy control animals were found clinically normal without any gross pathological changes in the mammary glands.

4.7.4.4 Ninety-six hours post challenge

After 96 h of challenge, Group II animals were found in depression and lachrymal discharges from eyes but not much gross pathological changes detected. No animals were found dead. The challenged mammary glands of herbal extract and honey treated animals (Group III, Group IV, Group V and Group VI) did not show much gross pathological changes, while Group VII antibiotic treated animals remained alert and active. The unchallenged Group I, healthy control animals were found clinically normal without any appreciable gross pathological changes in the mammary glands.

4.7.4.5 One hundred forty four hours post challenge

After 144 h of challenge, Group II animals were found dull but not much gross pathological changes detected. No animals were found dead. The challenged mammary glands of herbal extract and honey treated animals (Group III, Group IV, Group V and Group VI)

did not show much gross pathological changes, while Group VII antibiotic treated animals remained alert and active. The unchallenged Group I, healthy control animals were found clinically normal without any appreciable gross pathological changes in the mammary glands.

4.7.5 Total clinical scores (TCS) in response to herbal extracts and antibiotic therapy

The TCS remained 0.00±0.00 at 0 h, 24, 48 h, 96 h and 144 h of observational period in Group I mice. In Group II infected untreated control animals the total clinical scores ranged from 0.00±0.00 to 3.00±0.00 at 0 h, 24, 48 h, 96 h and 144 h. The TCS increased ($P<0.05$) significantly to an extent of 8.33% at 96 h and 144 h PC as compared to 24 h value in Group II animals. The experimental animals treated with herbal extracts + honey (Group III, Group IV, Group V and Group VI) and antibiotic treated Group VII revealed mild gross and pathological lesions when compared with infected animals of Group II. Whilst, *Piper longum*+ honey, *Listea monopetala* + honey and combination of *Glycyrrhiza glabra*, *Piper longum*, *Listea monopetala* and honey treated and antibiotic treated groups (Group IV, Group V, Group VI and Group VII) showed better response to therapy as compared to *Glycyrrhiza glabra* + honey treated group (Group III). However, Group IV, V, VI and VII did not differ much in their resistance towards *St. agalactiae* infection.

The TCS decreased ($P<0.05$) significantly to an extent of 22.22% in *Glycyrrhiza glabra* + honey treated Group III animals at 96 h and 144 h PC as compared to 0 h value. In Group IV *Piper longum* + honey treated mice, the scores decreased ($P<0.05$) significantly to an extent of 25.00% at 96 h and 144 h PC as compared to 0 h value. While, in *Listea monopetala* + honey treated Group V animals the total clinical scores decreased ($P<0.05$) significantly to an extent of 33.33% at 96 h and 144 h PC. In *Glycyrrhiza glabra*+ *Piper longum*+ *Listea monopetala* + honey treated Group VI animals the total clinical scores decreased ($P<0.05$) significantly to an extent of 44.44% at 96 h and 144 h PC as compared to 0 h value (Table 29 and Fig. 63).

The antibiotic treated Group VII revealed best response to treatment. In this group the total clinical scores decreased ($P<0.05$) significantly to an extent of 55.55% as compared to infected Group II animals at 96 h and 144 h PC. The general demeanor of antibiotic treated

Table 28: Total clinical scores (TCS) in experimental mice (Mean±SD) on day 0, 24, 48, 96 and 144 h post challenge

Groups	Time interval				
	0 h	24 h	48 h	96 h	144 h
I (Negative control)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
II (Positive control)	0.00±0.00	2.75±0.50^{B}	2.75±0.50^{B}	3.00±0.00^{B}	3.00±0.00^{B}
III (*Glycyrrhiza glabra* + honey)	0.00±0.00	2.25±0.50^{B}	2.25±0.50^{B}	1.75±0.50^{B}	1.75±0.50^{B}
IV (*Piper longum* + honey)	0.00±0.00	2.00±0.00^{B}	2.00±0.00^{B}	1.50±0.57^{B}	1.50±0.57^{B}
V (*Listea monopetala* + honey)	0.00±0.00	2.25±0.50^{B}	2.25±0.50^{B}	1.50±0.57^{A}	1.50±0.57^{A}
VI (*Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* + honey)	0.00±0.00	2.25±0.50^{B}	2.25±0.50^{B}	1.25±0.50^{B}	1.25±0.50^{B}
VII (Ciprofloxacin)	0.00±0.00	2.25±0.50^{C}	2.25±0.50^{C}	1.00±0.50^{B}	1.00±0.50^{B}

*Values with different superscripts are significant ($p<0.05$), in columns (A, B, C)

Table 29: Relative percentage of Lymphocytes and Neutrophils in mice of different groups at 0 h, 48 h, 96 h and 144 h (Mean±SD)

Groups	Lymphocytes				Neutrophils			
	0 h	48 h	96 h	144 h	0 h	48 h	96 h	144 h
I (Negative control)	79.50±1.04^{AX}	77.50±0.54^{DX}	78.83±1.47^{DX}	78.66±1.63^{DX}	15.33±0.51^{AX}	16.66±0.51^{AZ}	15.66±0.51^{AXY}	16.16±0.40^{AYZ}
II (Positive control)	78.83±1.60^{AZ}	41.50±1.37^{AY}	40.66±0.81^{AY}	33.16±1.94^{AX}	15.33±0.51^{AX}	50.33±0.51^{EY}	49.66±0.51^{FY}	53.83±0.51^{DZ}
III (*Glycyrrhiza glabra* + honey)	78.83±1.60^{AZ}	64.16±1.47^{BY}	63.00±0.89^{BY}	60.33±0.51^{BY}	16.16±0.40^{ABX}	29.16±0.40^{DZ}	27.83±0.40^{EY}	28.83±0.40^{CZ}
IV (*Piper longum* + honey)	78.66±1.63^{AZ}	74.50±0.54^{CY}	70.83±0.75^{CX}	79.00±0.89^{CX}	16.50±0.54^{BX}	19.33±0.51^{CY}	19.66±0.51^{DY}	19.50±0.54^{BY}
V (*Listea monopetala* + honey)	78.66±1.63^{AZ}	74.16±1.16^{CY}	72.33±0.51^{CXY}	71.16±1.16^{CX}	16.83±0.40^{BX}	19.50±0.54^{CZ}	18.66±0.51^{BCY}	19.50±0.54^{BZ}
VI (*Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* + honey)	78.66±1.63^{AZ}	75.66±1.21^{CDY}	72.33±0.51^{CX}	71.00±1.26^{CX}	16.16±0.40^{ABX}	18.16±0.40^{BY}	17.83±0.40^{BY}	18.83±0.50^{BZ}
VII (Ciprofloxacin)	78.50±1.64^{AZ}	75.16±0.75^{CY}	72.33±0.51^{CX}	72.33±1.63^{CX}	16.83±0.40^{BX}	17.50±0.54^{BCX}	19.50±0.54^{CDY}	19.33±0.75^{BY}

*Values with different superscripts are significant at level ($p<0.05$), in rows (A, B, C, D, E, F) and column (X, Y, Z)

animals improved considerably after therapy. After 24 h of antibiotic treatment all the animals showed improvement in general condition. After 48 h of treatment marked reduction in signs of inflammation was evident. At 144 h, the animals appeared normal without any evidence of clinical signs (Table 28 and Fig. 63).

4.8 Differential leukocyte count (DLC)

Differential leukocyte count of experimental mice is presented in Table 29 and Table 30. In mouse lymphocytes are more in number than neutrophils. The relative percentage of lymphocytes ranged from 77.50±0.54% to 79.50±1.04% in Group I healthy mice. The relative percentage of lymphocytes decreased ($P<0.05$) significantly in all the infected groups (Group III, Group IV, Group V, Group VI and Group VII) as compared to healthy group (Group I) at 48h, 96 h and 144 hr (Table 29 and Fig. 64).

The relative percentage of lymphocytes decreased ($P<0.05$) significantly in infected untreated mice of Group II to an extent of 47.35% , 48.42% and 57.93% at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. While, relative percentage of lymphocytes decreased ($P<0.05$) significantly to 57.84% as compared to Group I healthy mice at 144 h (Table 29 and Fig. 64).

In *Piper longum* + honey treated Group III mice the relative percentage of lymphocytes decreased ($P<0.05$) significantly to an extent of 18.60% , 20.08% and 23.46% at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. While, relative percentage of lymphocytes decreased ($P<0.05$) significantly to 23.30% as compared to Group I healthy mice at 144h (Table 29 and Fig. 64).

In *Glycyrrhiza glabra* + honey treated Group IV mice the relative percentage of lymphocytes decreased ($P<0.05$) significantly to an extent of 5.28% , 9.95% and 11% at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. However, relative percentage of lymphocytes decreased ($P<0.05$) significantly to 11% as compared to Group I healthy mice at 144 h (Table 29 and Fig. 64).

The relative percentage of lymphocytes decreased ($P<0.05$) significantly in *Listea monopetala* + honey treaed Group V to an extent of 5.72% , 8.04% and 9.53% at 48 h, 96

h and 144 h PC respectively as compared to 0 h value. Whilst, relative percentage of lymphocytes decreased ($P<0.05$) significantly to 9.53% as compared to Group I healthy mice at 144 h (Table 29 and Fig. 64).

In *Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* + honey treated Group VI mice the relative percentage of lymphocytes decreased ($P<0.05$) significantly to an extent of 3.81% , 8.04% and 9.73% at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. Whilst, relative percentage of lymphocytes decreased ($P<0.05$) significantly to 9.73% as compared to Group I healthy mice at 144 h (Table 29 and Fig. 64).

The relative percentage of lymphocytes decreased ($P<0.05$) significantly in Group VII to an extent of 4.25% , 7.85% and 7.85% at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. Whilst, relative percentage of lymphocytes decreased ($P<0.05$) significantly to 8.04% as compared to Group I healthy mice at 144 h (Table 29 and Fig. 64).

The relative percentage of neutrophils ranged from 15.33±0.51% to 16.66±0.51% in Group I healthy mice (Table 29). Significantly ($P<0.05$) higher neutrophil levels were recorded in all the infected groups as compared to healthy group (Group I) at 48h, 96 h and 144 h . However, infected untreated Group II animals recorded significantly ($P<0.05$) higher percentage of neutrophils than other infected groups (Group III, Group IV, Group V, Group VI and Group VII).

Group II animals recorded an increase of 69.54%, 69.13% and 71.52% in the relative percentage of neutrophils at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. The number of neutrophils increased significantly ($P<0.05$) to 69.97% as compared to Group I healthy mice at 144 h (Table 29 and Fig. 65).

Glycyrrhiza glabra + honey treated Group III animals recorded significant ($P<0.05$) increase of 45.13% , 42.50% and 44.50% in the number of neutrophils at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. The number of neutrophils increased significantly ($P<0.05$) to 43.94% as compared to Group I healthy mice at 144 h (Table 29 and Fig. 65).

Piper longum + honey treated group IV animals recorded significant ($P<0.05$) increase of 14.64% , 16.07% and 15.38% in the number of neutrophils at 48 h, 96 h and 144 h PC

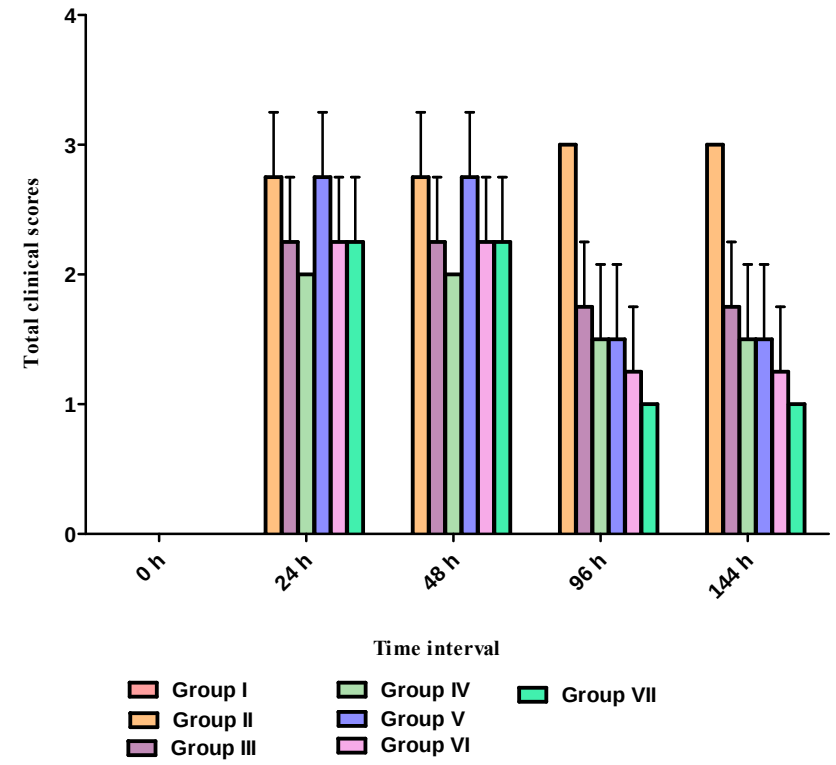


Fig. 63: Total clinical scores in experimental mice

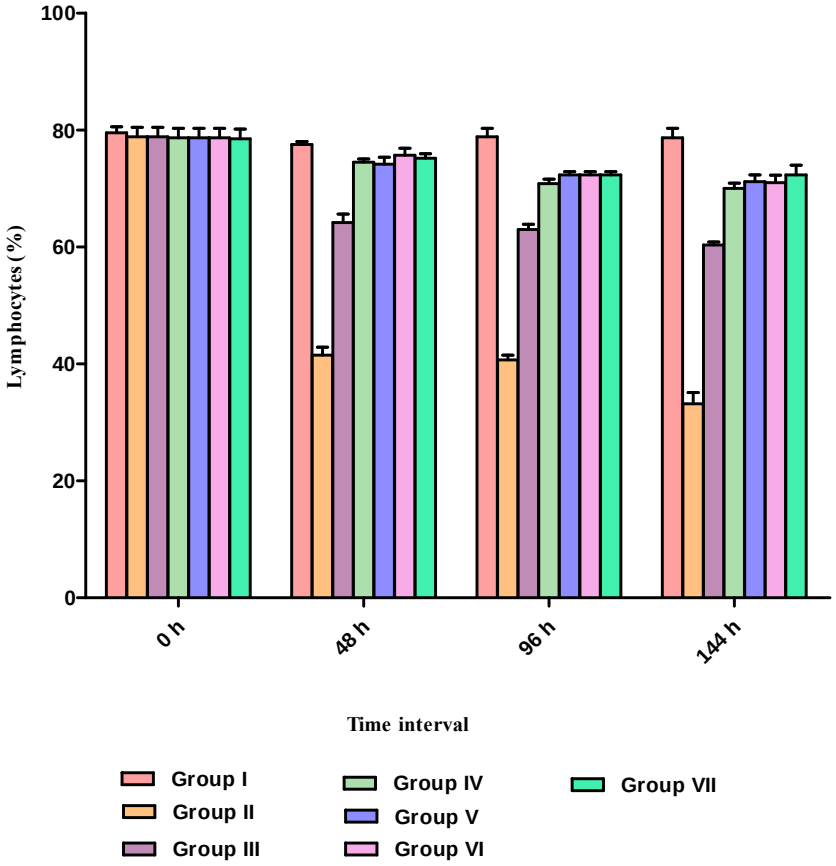


Fig. 64: Relative percentage of lymphoctes in mice of different groups

respectively as compared to 0 h value. The number of neutrophils increased significantly ($P<0.05$) to 17.12% as compared to Group I healthy mice at 144 h (Table 29 and Fig. 65).

Listea monopetala+ honey treated treated Group V animals recorded significant ($P<0.05$) increase of 13.69% , 9.80% and 13.69% in the number of neutrophils at 48 h, 96 h and 144 h PC as compared to 0 h value. The number of neutrophils increased significantly ($P<0.05$) to 17.12% as compared to Group I healthy mice at 144 h (Table 29 and Fig. 65).

In *Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* + honey treated Group VI animals, the relative percentage of neutrophils increased significantly ($P<0.05$) to an extent of 11.01% , 9.36% and 14.17% at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. The number of neutrophils increased significantly ($P<0.05$) to 14.17% as compared to Group I healthy mice at 144 h (Table 29 and Fig. 65).

In Ciprofloxacin treated Group VII animals, the relative percentage of neutrophils increased significantly ($P<0.05$) to an extent of 3.82% , 13.69% and 12.93% at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. The number of neutrophils increased significantly ($P<0.05$) to 16.39% as compared to Group I healthy mice at 144 h (Table 29 and Fig. 65).

The number of monocytes ranged from 3.66±0.51% to 6.33±0.51% at 0 h, 48 h, 96 h and 144 h in Group II animals. The number of monocytes increased ($P<0.05$) significantly in Group II infected animals at 48h, 96 h and 144 h PC to an extent of 4.43%, 35.33% and 42.18%as compared to 0 h value. However, the relative percentage of monocytes increased ($P<0.05$) significantly to 44.70% as compared to Group I animals at 144 h (Table 30 and Fig. 66).

The number of monocytes increased ($P<0.05$) significantly in all the treated groups (Group III, Group IV, Group V, Group VI) as compared to the healthy Group I animals at 48 h, 96 h and 144 h PC. *Glycyrrhiza glabra* + honey treated Group III animals recorded significant ($P<0.05$) increase of 15.47%, 29.06 % and 31.33% in the number of monocytes at 96 h and 144 h PC respectively as compared to 0 h value. The number of monocytes

increased significantly (P<0.05) to 34.33% as compared to Group I healthy mice at 144 h (Table 30 and Fig. 66).

The relative percentage of monocytes increased significantly (P<0.05) in *Piper longum*+ honey treated Group IV animals to an extent of 23.09%, 37.52% and 42.88% at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. The number of monocytes increased significantly (P<0.05) to 39.96% as compared to Group I healthy mice at 144 h (Table 30 and Fig. 66).

Listea monopetala + honey treated Group V animals recorded significant (P<0.05) increase of 15.47%,31.33% and 37.22% in the number of monocytes at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. The number of monocytes increased significantly (P<0.05) to 39.96% as compared to Group I healthy mice at 144 h (Table 30 and Fig. 66).

In *Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* + honey treated Group VI animals, the relative percentage of monocytes increased significantly (P<0.05) to an extent of 19.95%, 41.16% and 42.88% at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. The number of monocytes increased significantly (P<0.05) to 39.96% as compared to Group I healthy mice at 144 h (Table 30 and Fig. 66).

Antibiotic treated Group VII animals recorded significant (P<0.05) increase of 24.89%, 27.53% and 34.33% in the number of monocytes at 48 h, 96 h and 144 h PC as compared to 0 h value. The number of monocytes increased significantly (P<0.05) to 34.33% as compared to Group I healthy mice at 144 h (Table 30 and Fig. 66).

There was no significant difference in the eosinophil and basophil counts of infected groups as compared to healthy group at 48 h, 96 h and 144 h PC (Table 30).

4.9 C-reactive protein (CRP)

The semi-qualitative analysis of C reactive protein was performed in the serum of experimental animals and the results of the analysis are presented in Table 31. The results of qualitative analysis were ranged from negative (-) to positive (+) (Fig. 67). The serum samples of Group I animals were CRP negative during throughout the study at 0 h, 24 h, 48 h and 96 h PC.

Table 30: Relative percentage of Monocytes, Eosinophils and Basophils in mice of different groups at 0 h, 48 h, 96 h and 144 h (Mean±SD)

Groups	Monocytes				Eosinophils				Basophils			
	0 h	48 h	96 h	144 h	0 h	48 h	96 h	144 h	0 h	48 h	96 h	144 h
I (Negative control)	3.50^{AX} ±0.54	3.66^{AX} ±0.51	3.50^{AX} ±0.54	3.50^{AX} ±0.54	1.16^{AX} ±0.75	1.66^{AX} ±0.51	1.16^{AX} ±0.75	1.00^{AX} ±0.63	0.50^{AX} ±0.54	0.66^{AX} ±0.51	0.83^{BX} ±0.40	0.83^{BX} ±0.40
II (Positive control)	3.66^{AX} ±0.51	3.83^{AX} ±0.98	5.66^{BY} ±0.51	6.33^{CY} ±0.51	2.33^{BX} ±0.51	2.66^{BX} ±0.51	4.16^{CY} ±0.40	5.33^{CZ} ±0.51	0.33^{AX} ±0.51	0.66^{AXY} ±0.51	1.00^{BX} ±0.00	1.16^{BY} ±0.40
III (*Glycyrrhiza glabra* + honey)	3.66^{AX} ±0.51	4.33^{AXY} ±0.51	5.16^{BY} ±0.75	5.33^{BZ} ±0.51	0.83^{AX} ±1.50	1.50^{AX} ±0.54	3.16^{BY} ±0.40	4.66^{BCZ} ±0.51	0.66^{AX} ±0.51	0.83^{AX} ±0.40	0.83^{BX} ±0.40	0.83^{BX} ±0.40
IV (*Piper longum* + honey)	3.33^{AX} ±0.51	4.33^{AY} ±0.81	5.33^{BZ} ±0.51	5.83^{BCZ} ±0.40	0.83^{AX} ±0.75	1.50^{AX} ±0.54	3.33^{BCY} ±0.51	3.83^{BCY} ±0.40	0.50^{AX} ±0.54	0.50^{AX} ±0.54	0.83^{BX} ±0.40	0.83^{BX} ±0.40
V (*Listea monopetala* + honey)	3.66^{AX} ±0.51	4.33^{AX} ±0.81	5.33^{BY} ±0.51	5.83^{BCY} ±0.40	0.66^{AX} ±0.51	1.50^{AX} ±0.54	3.66^{BCY} ±0.51	3.66^{BCY} ±0.51	0.16^{XY} ±0.40	0.66^{AY} ±0.51	0.00^{AX} ±0.00	0.00^{AX} ±0.00
VI (*Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* + honey)	3.33^{AX} ±0.51	4.16^{AY} ±0.75	5.66^{BZ} ±0.81	5.83^{BCZ} ±0.75	1.16^{AX} ±0.75	1.50^{AX} ±0.54	3.50^{BCY} ±0.54	3.83^{BCY} ±0.75	0.83^{AX} ±0.40	0.66^{AX} ±0.51	0.66^{BX} ±0.51	0.66^{AX} ±0.51
VII (Ciprofloxacin)	3.50^{AX} ±0.54	4.66^{AY} ±0.51	4.83^{BY} ±0.75	5.33^{BY} ±0.51	0.66^{AX} ±0.51	1.50^{AX} ±0.54	3.33^{BCY} ±0.51	3.00^{BY} ±0.89	0.40 ±0.54^{XY}	0.83^{AY} ±0.40	0.00^{AX} ±0.00	0.00^{AX} ±0.00

*Values with different superscripts are significant at level ($p<0.05$), in rows (A, B, C) and column (X, Y, Z)

Table 31: Qualitative analysis of CRP in response to methanolic extracts of *Glycyrrhiza glabra* + honey, *Piper longum* + honey, *Listea monopetala* + honey, *Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* + honey and Ciprofloxacin at day 0 h, 24 h, 48 h and 96 hours post challenge in experimental mice

Group	Mouse number	0 h	24 h	48 h	96 h
I (Negative control)					
	1	Negative	Negative	Negative	Negative
	2	Negative	Negative	Negative	Negative
	3	Negative	Negative	Negative	Negative
	4	Negative	Negative	Negative	Negative
	5	Negative	Negative	Negative	Negative
	6	Negative	Negative	Negative	Negative
II (Positive control)					
	1	Negative	Positive(+)	Positive(+)	Negative
	2	Negative	Positive(+)	Positive(+)	Negative
	3	Negative	Positive (+)	Positive(+)	Negative
	4	Negative	Positive(+)	Positive(+)	Negative
	5	Negative	Positive(+)	Positive(+)	Negative
	6	Negative	Negative	Negative	Negative
III (*Glycyrrhiza glabra* + honey)					
	1	Negative	Positive(+)	Positive(+)	Negative
	2	Negative	Negative	Negative	Negative
	3	Negative	Negative	Negative	Negative
	4	Negative	Positive(+)	Positive(+)	Negative
	5	Negative	Negative	Negative	Negative
	6	Negative	Negative	Negative	Negative
IV(*Piper longum* + honey)					
	1	Negative	Positive(+)	Positive(+)	Negative
	2	Negative	Positive(+)	Positive(+)	Negative
	3	Negative	Negative	Negative	Negative
	4	Negative	Negative	Negative	Negative
	5	Negative	Negative	Negative	Negative
	6	Negative	Negative	Negative	Negative
V(*Listea monopetala* + honey)					
	1	Negative	Negative	Negative	Negative
	2	Negative	Negative	Negative	Negative
	3	Negative	Negative	Negative	Negative
	4	Negative	Positive(+)	Positive(+)	Negative
	5	Negative	Negative	Negative	Negative
	6	Negative	Negative	Negative	Negative

Table 31: Contd...

Group	Mouse number	0 h	24 h	48 h	96 h
VI (*Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* + honey)					
	1	Negative	Negative	Negative	Negative
	2	Negative	Positive(+)	Positive(+)	Negative
	3	Negative	Negative	Negative	Negative
	4	Negative	Negative	Negative	Negative
	5	Negative	Negative	Negative	Negative
	6	Negative	Negative	Negative	Negative
VII (Ciprofloxacin)					
	1	Negative	Negative	Negative	Negative
	2	Negative	Negative	Negative	Negative
	3	Negative	Negative	Negative	Negative
	4	Negative	Positive(+)	Positive(+)	Negative
	5	Negative	Negative	Negative	Negative
	6	Negative	Negative	Negative	Negative

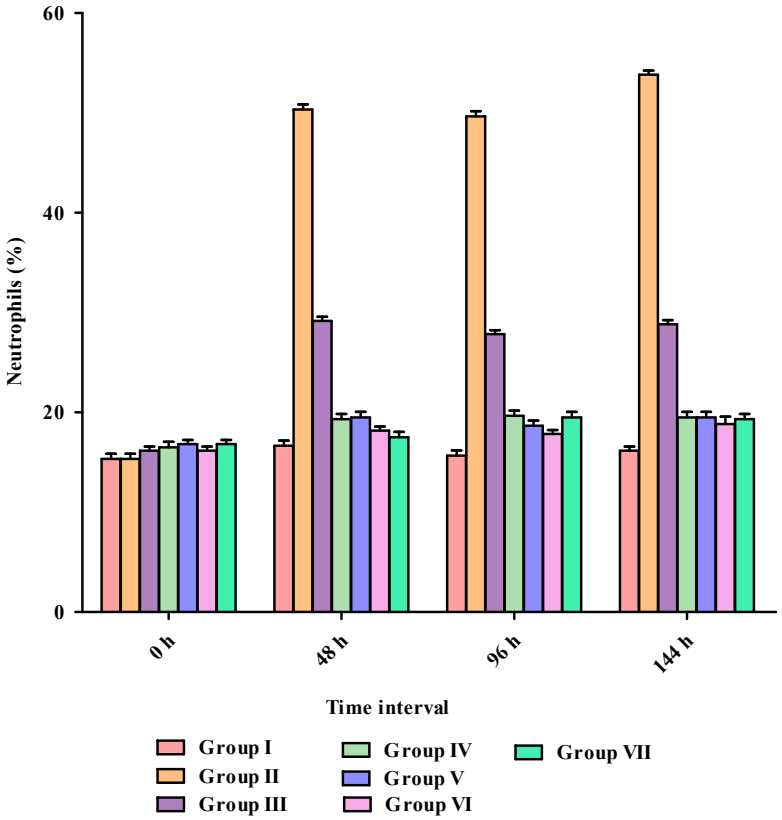


Fig. 65: Relative percentage of neutrophils in mice of different groups

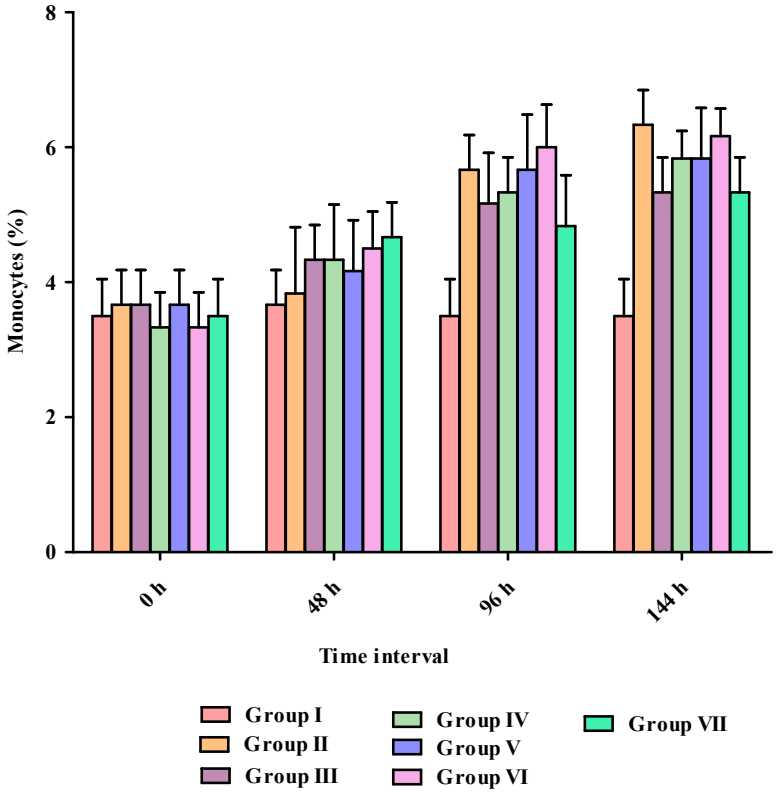


Fig. 66: Relative percentage of monocytes in mice of different groups

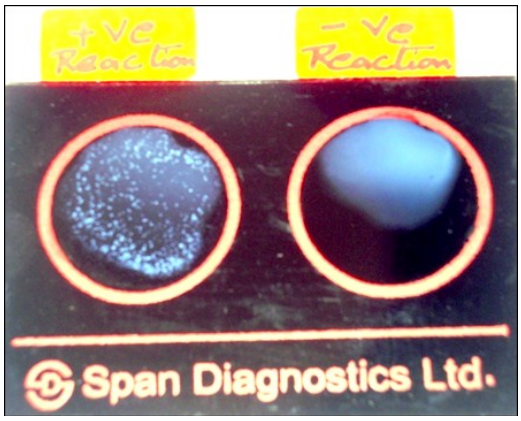


Fig. 67: Positive and negative CRP agglutination reaction at 24 h and 96 h in experimental group

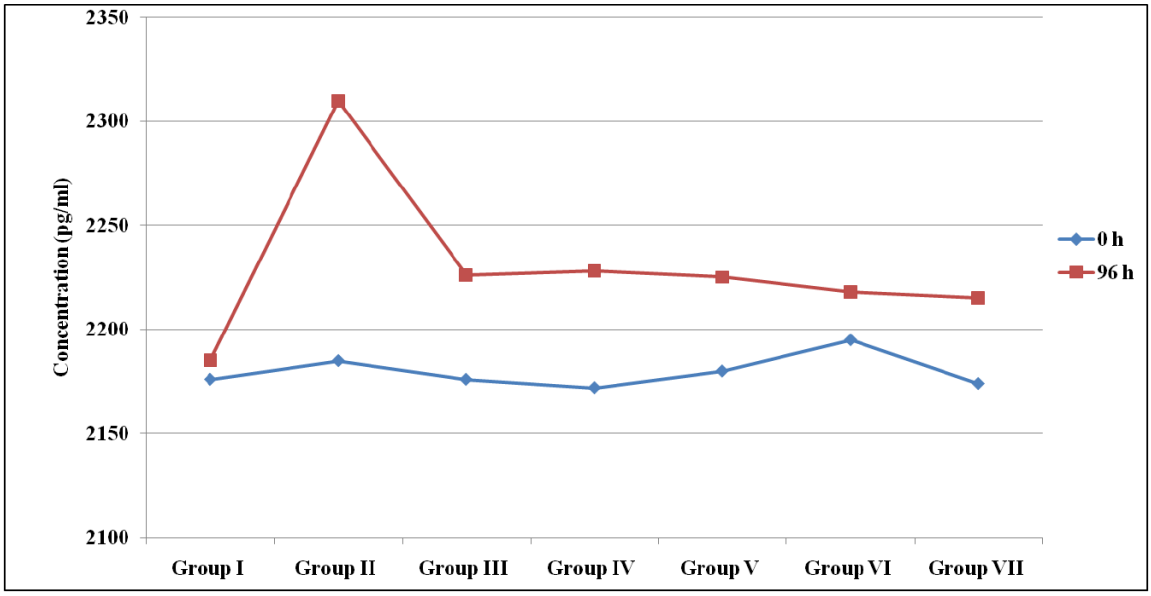


Fig. 68: Graph showing Concentration of IL-12P40 (pg/ml) in response to therapy in mice at 0 h and 96 h

The C reactive protein in animals of Group II *i.e.* infected control ranged from negative (-) at 0 h to positive (+) at 48 h PC. All the animals from Group II were positive at 24 h and 48 h PC after that at 96 h agglutination reaction was negative. Two animals from Group III and IV group revealed presence of CRP in their blood serum at 24 and 48 h. Thereafter, the acute phase reaction diminished. (Table 31).

Animals from Group V, VI and VII revealed almost similar results and one animal from these groups showing presence of CRP in their blood serum at 24 and 48 h. Thereafter, the level of CRP diminished drastically at 96 h PC (Table 31).

4.10 Concentration of IL-12p40 (pg/ml) in response to therapy in mice

Serum level of IL-12 p40 was significantly increased in positive control Group II animal (2310 pg/ml) as compared to negative control Group I healthy animal (2185 pg/ml) at 96 h. In antibiotic treated Group VII animal its level was low (2215 pg/ml) followed by Groups VI (2218 pg/ml), Group V (2225 pg/ml) Group IV (2228 pg/ml) and Group III (2226 pg/ml) animal at 96 h (Table 32 & Fig. 68). In Group I animals there was no increase in concentration of IL-12 p40 at 96 h from 0 h value.

4.11 Histopathological examination of mammary tissue

The mice were sacrificed at 144 hours post challenge and mammary glands were harvested for histopathological examination.The mice were anaesthetized with ketamine (100 mg/ kg) and xylazine (10mg/kg) before sacrifice. The following results were obtained on histopathological examination of different groups.

4.11.1 Group I

Tissue sections of mammary gland stained with H & E stain revealed normal healthy lactating alveoli filled with milk. No Inflammatory reactions were observed in the section (Fig. 69). The distended alveoli revealed eosinophilic contents reducing the interlobular contents to thin septa between lobules.

4.11.2 Group II

The histopathological examination revealed severe inflammatory changes, characterized by cellular infiltration with distinct neutrophils and disruption of normal architecture of the

gland. Necrosis of mammary gland tissue was noticed in the section. Diffused necrotic foci with complete disruption of cellular architecture were observed (Fig. 70).

4.11.3 Group III

Tissue sections of mammary gland revealed distended and non- distended alveoli with mild inflammatory reaction as compared to the infected untreated control. Besides, cellular architecture was not completely lost. The fibrocellular reaction around the necrosed mammary gland alveoli in the mammary gland tissue sections was less as compared to the infected untreated control (Fig. 71).

4.11.4 Group IV

Tissue sections of mammary gland revealed comparatively reduced inflammatory reaction with only mild pathological changes as compared infected untreated control animals of Group II. Alveoli were not in healthy condition but necrosis was there and inflammatory changes in interstitial tissue showing elongated fibroblast with neutrrophils (Fig. 72).

4.11.5 Group V

Tissue sections of mammary gland revealed both non-distended and distended alveoli with scanty inflammatory exudate of mononuclear cells, proliferation of interstitial tissue and cellular hyperplasia However, tissue architecture appeared almost normal (Fig. 73).

4.11.6 Group VI

Tissue sections revealed non-distended alveoli with moderate mononuclear cell infiltration and fibrosis. These changes were mild in severity than that of Group III animals. Interstitial tissue showed necrosed area with healthy and weak alveoli epithelium (Fig. 74).

4.11.7 Group VII

Tissue sections appeared almost normal with mild inflammatory reaction in comparison to any other treated group. There was mild fibrous tissue reaction with cellular infiltration (Fig. 75).

4.12 Demonstration of bacteria in mammary gland tissue sections of infected untreated control group animals

Mammary gland tissue sections revealed the presence of gram positive cocci indistinguishable from *St. agalactiae* on Gram's (Fig. 76) and Giemsa staining (Fig. 77).

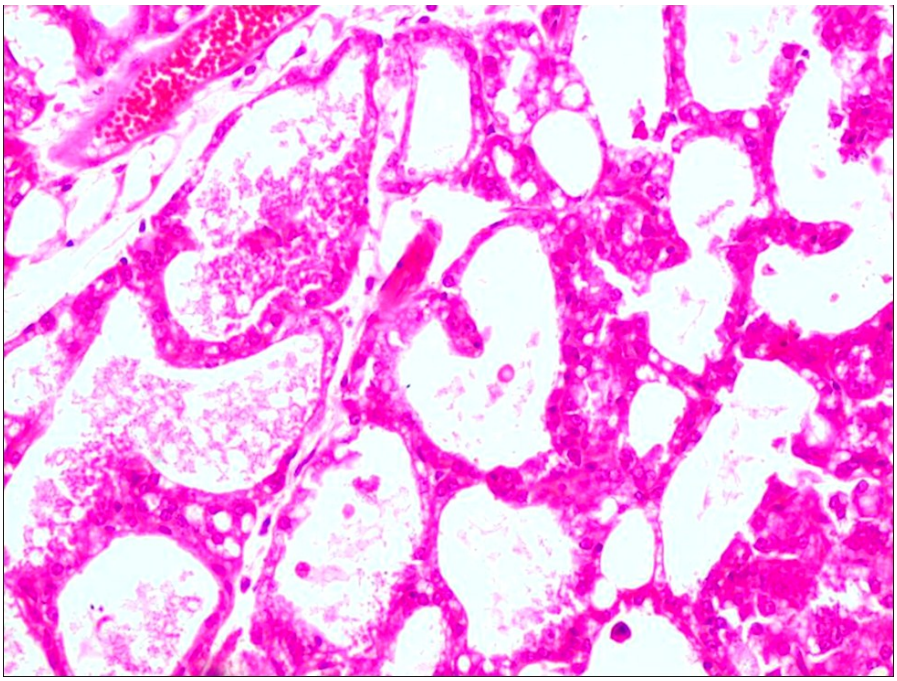


Fig. 69: Mammary gland showing normal lactating acini in Group I healthy/ negative control mice

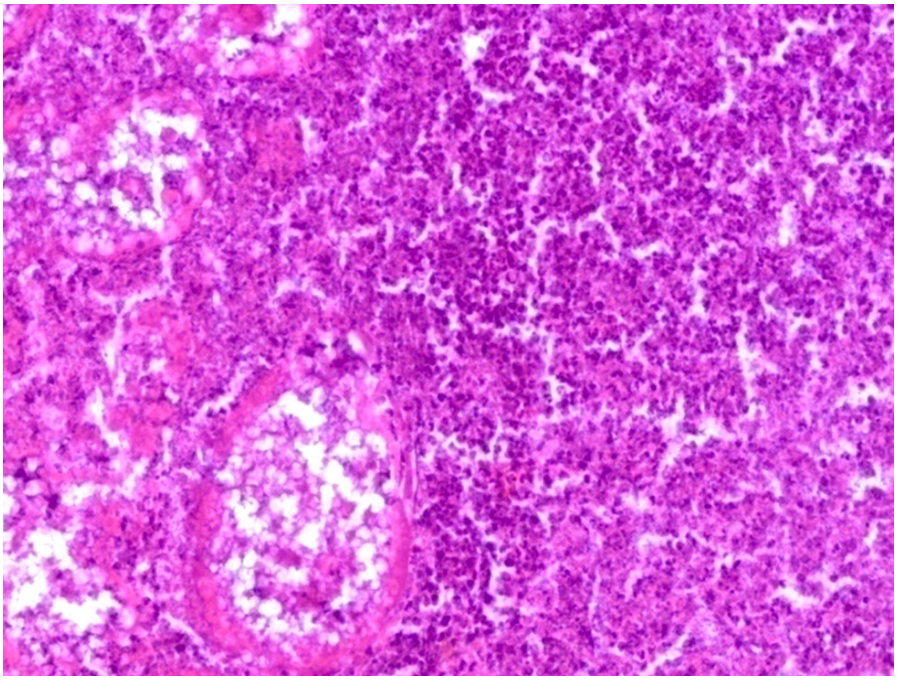


Fig. 70: Mammary gland showing severe PMN cellular infiltrations with complete loss of cellular architecture in Group II infected/positive control mice

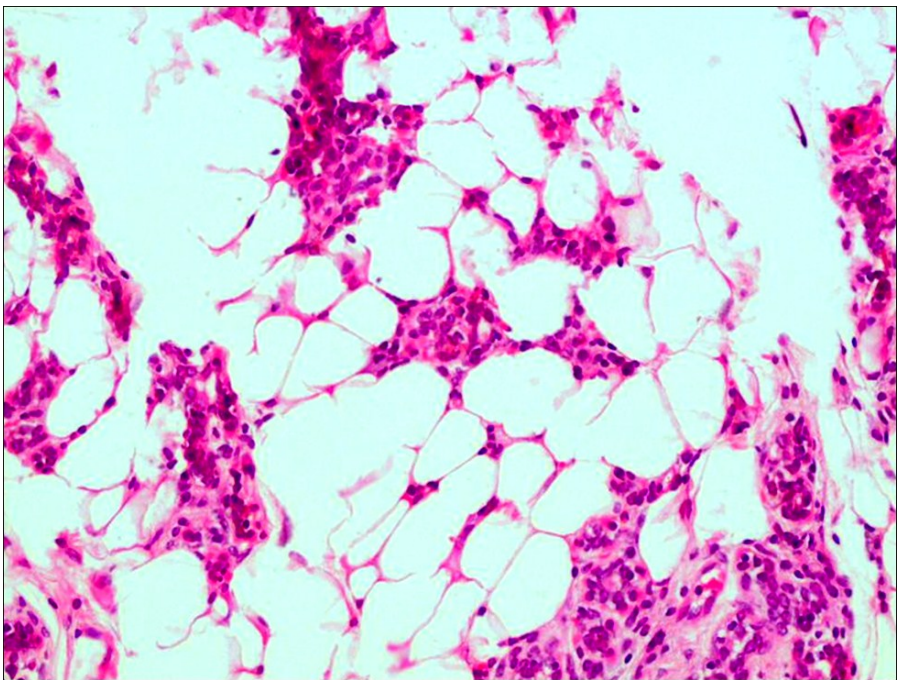


Fig. 71: Mammary gland showing moderate inflammatory cellular infiltration with only partial loss of architecture in group III mice drenched with *Glycyrrhiza glabra* + honey and challenged with *St. agalactiae*

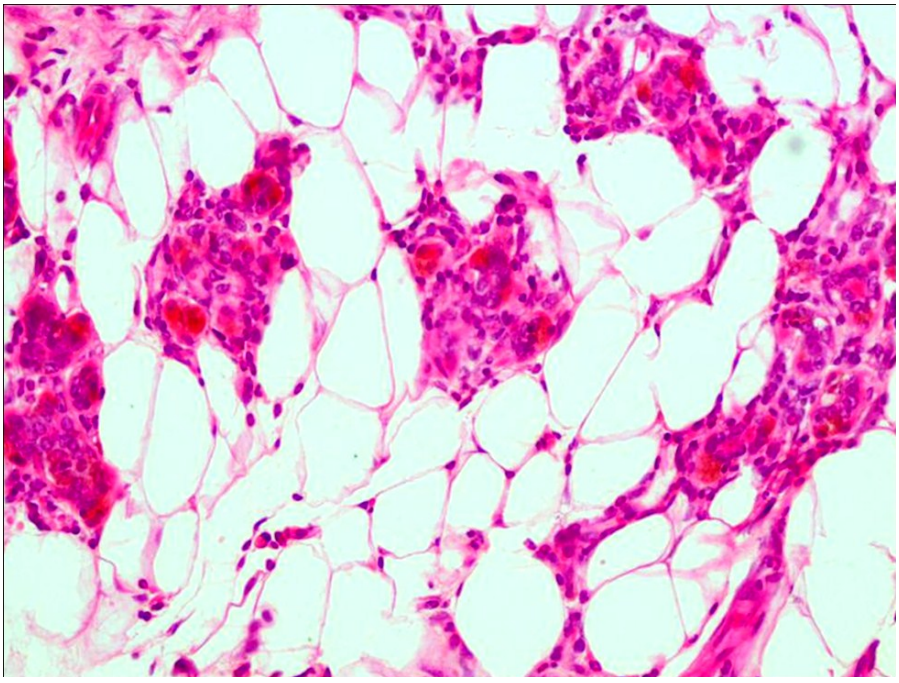


Fig. 72: Mammary gland showing moderate inflammatory cellular infiltration with only partial loss of architecture in group IV mice drenched with *Piper longum* + honey and challenged with *St. agalactiae*

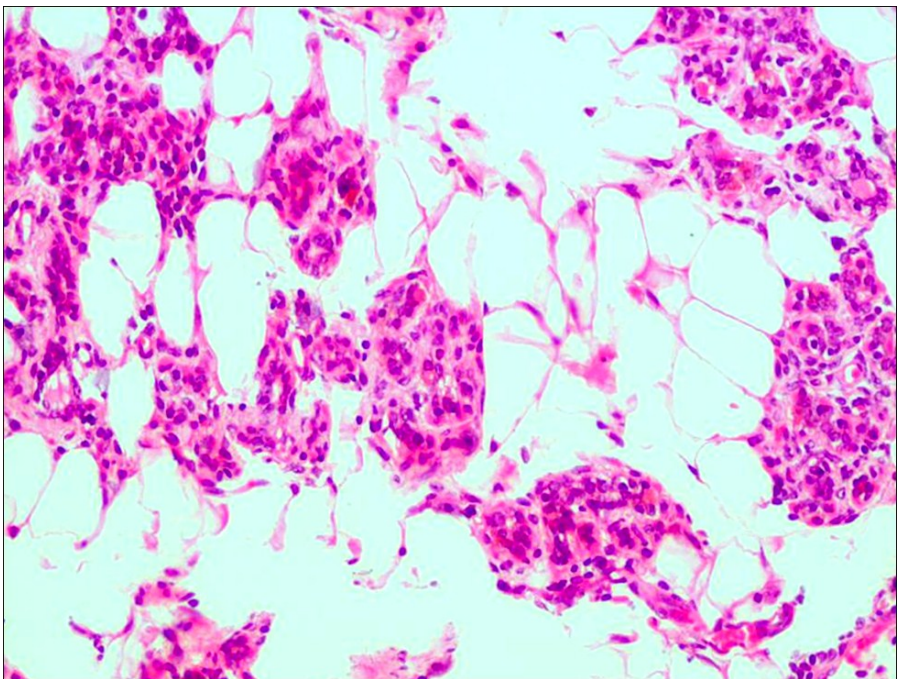


Fig. 73: Mammary gland showing moderate inflammatory cellular infiltration with only partial loss of architecture in group V mice drenched with *Listea monopetala* + honey and challenged with *St. agalactiae*

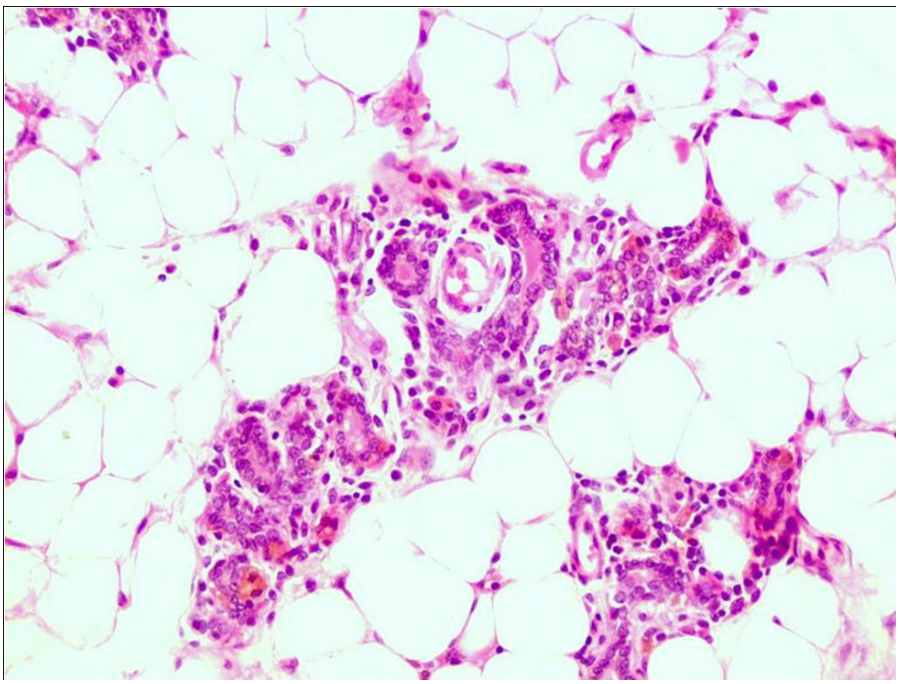


Fig. 74: Mammary gland showing moderate inflammatory cellular infiltration with only partial loss of architecture in group VI mice drenched with *Glycyrrhiza glabra* + *Piper longum*+ *Listea monopetala* + honey and challenged with *St. agalactiae*

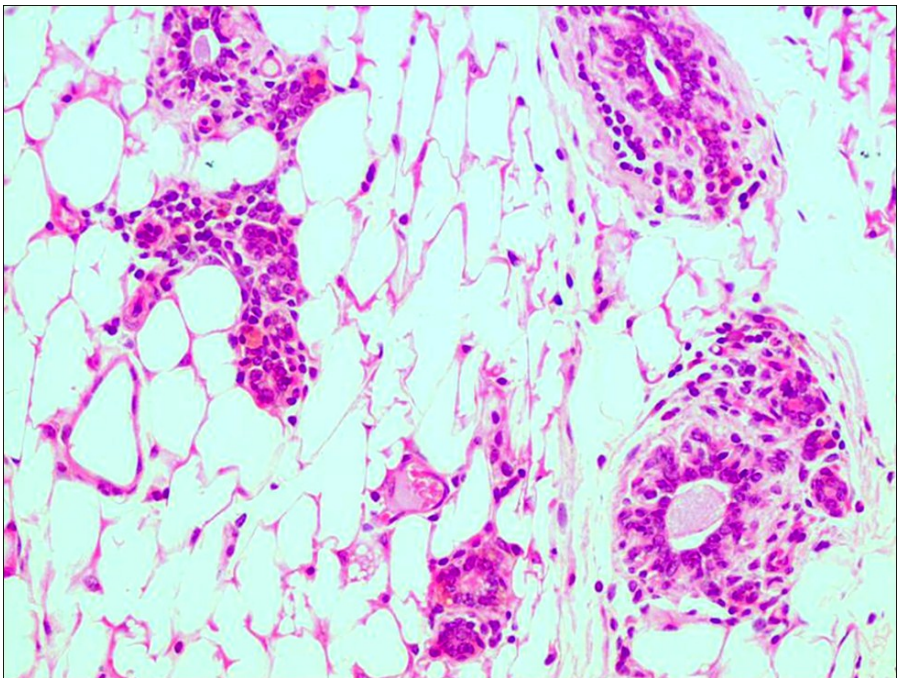


Fig. 75: Mammary gland showing moderate inflammatory cellular infiltration with only partial loss of architecture in Group VII mice drenched with Ciprofloxacin and challenged with *St. agalactiae*

4.13 Bacterial isolation and bacterial counts in mammary gland

L-4 mammary glands from two mice from each group were triturated with 1ml sterile normal saline or PBS in a sterile pestle and mortar. The triturate was then inoculated on to nutrient agar plates. After 16-18 h of incubation at 37°C, the colonies were counted by colony counter (Fig. 78). No viable organism could be isolated from the mammary glands of healthy control. In the infected untreated group (Group II), high mean bacterial count of 6.40×10^6 was obtained at 144 h PC. Mean viable count in Group III mice was 3.80×10^4. In Group IV animals mean viable count of 3.85×10^4 was observed. In Group V mean viable count was 3.70×10^4. In mice of Group VI mean viable count of 3.60×10^4 was observed. In antibiotic treated Group VII mean viable count of 3.00×10^3 was obtained (Table 33). The viable organisms recovered from mammary glands of group III, IV, V and VI revealed reduction in viable counts with antibiotic treated group revealing the lowest number of viable organisms.

Table 32: Concentration of IL-12p40 (pg/ml) in response to therapy in mice

Groups	Concentration of IL-12p40 (pg/ml)	
	0 h	96 h
I (Negative control)	2176	2185
II (Positive control)	2185	2310
III (*Glycyrrhiza glabra* + honey)	2176	2226
IV (*Piper longum* + honey)	2172	2228
V (*Listea monopetala* + honey)	2180	2225
VI (*Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* + honey)	2195	2218
VII (Ciprofloxacin)	2174	2215

Table 33: Mean viable count of *St. agalactiae* in mammary gland tissue of mice after 144 h

Groups	Mean viable count of *St. agalactiae*
I (Negative control)	0
II (Positive control)	6.40×10^6
III (*Glycyrrhiza glabra* + honey)	3.80×10^4
IV (*Piper longum* + honey)	3.85×10^4
V (*Listea monopetala* + honey)	3.70×10^4
VI (*Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* + honey)	3.60×10^4
VII (Ciprofloxacin)	3.00×10^3

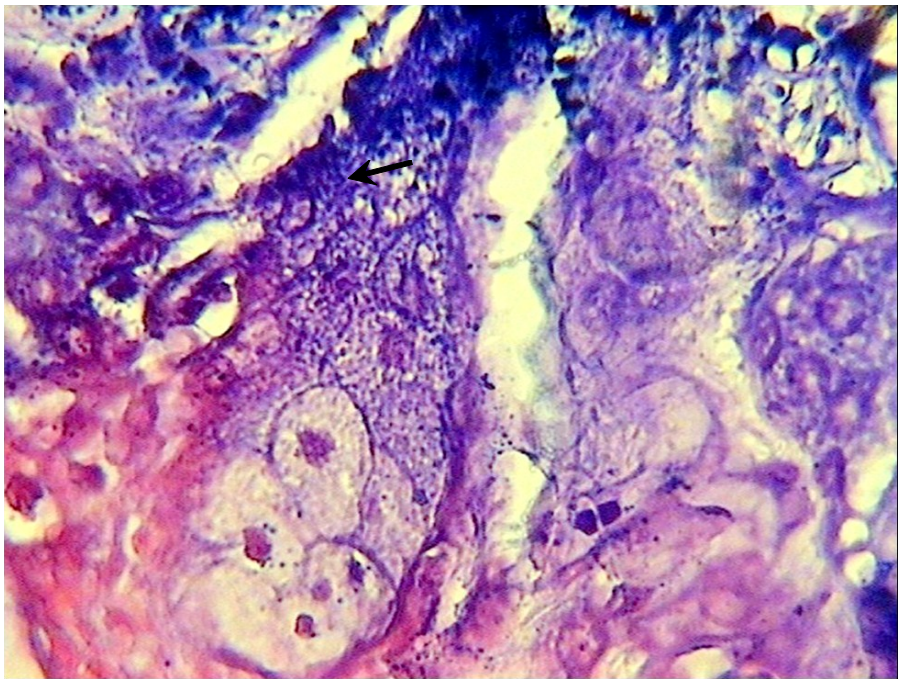


Fig. 76: Mammary gland showing presence of cocci (Gram's staining)

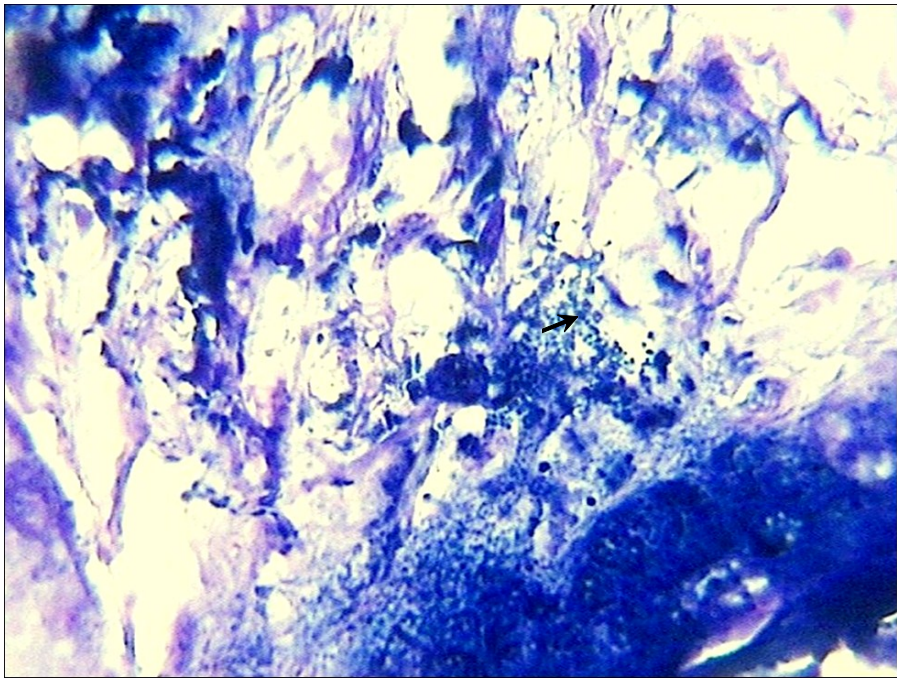


Fig. 77: Mammary gland showing presence of cocci (Geimsa staining)

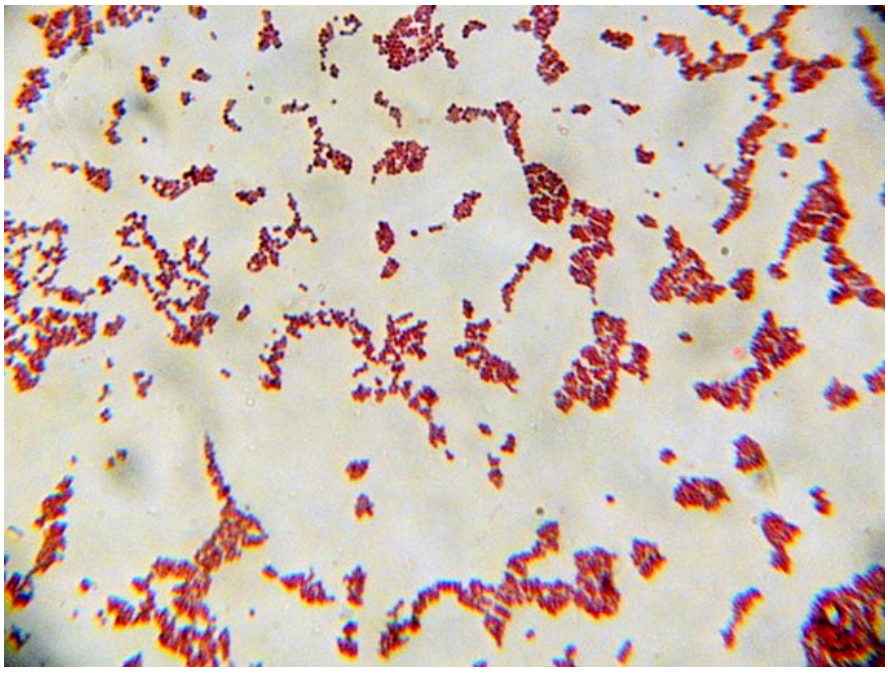
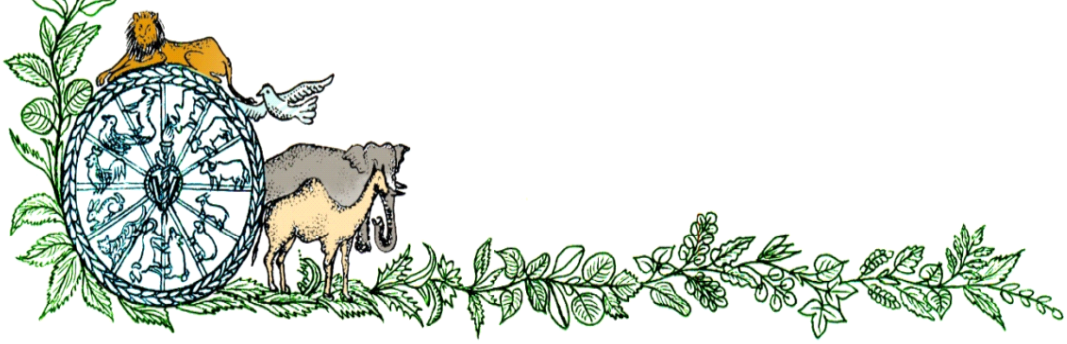


Fig. 78: Gram positive cocci (*St. agalactiae*) in mammary gland culture isolate (Gram's staining)

Discussion



5 DISCUSSION

5.1 Ethno-veterinary medicine

Ethno-veterinary medicine (EVM) or practices (EVP) covers people's knowledge, skills, methods, practices and beliefs about the care of their animals (McCorkle, 1986). Jharkhand has a high density of flora with plants rich in high medicinal values. The trend of treating illnesses with indigenous ayurvedic system is several years old, which the adivasi community has, inherited it from their ancestors. The forest area is about 40% of the total area of Jharkhand. 32 tribal communities found in Jharkhand (Lal and Singh, 2012).Traditional knowledge is in danger of extinction because there has been little documentation of traditional knowledge, rather it has been transmitted across generations by orally (Matekaire and Bwakura, 2004). Documentation is the first step towards effective utilization of EVM. Recently there are some studies done in India for gastrointestinal disorders and general health care management (Balakrishnan *et al*., 2009). Some ethnoveterinary medicines used by tribe Paniyars in Kerala, India for general health conditions as reported by Thomas *et al*. (2011). Ethnoveterinary plants of district Hamirpur for cure of livestock used by rural populace of Himachal Pradesh, India documented by (Sehgal and Sood, 2013). Animal health care management practices among the tribal community of Malda district of West Bengal, India reported by Saha *et al*. (2014). But still very little information is collected and documented. Present study was planned in this context to document the existing EVPs in rural areas of West Singhbhum district, Jharkhand. Participatory Rural Appraisal (PRA) was the tools used for the present study. Interviews, field visits and focused group discussions were used as the tools of PRA. The

information was collected using pre-designed questionnaires and open-ended interviews. Focused group discussions were arranged to verify the information provided by the key respondents to reach more accurate results (Santhanakrishnan *et al*., 2008; Lans *et al*., 2000; Dilshad *et al*., 2008). Ethno-veterinary Medicine is used for the maintenance of good animal health in developing countries (UNESCO, 1996 cited by Kudi, 2003). According to an estimate over 80% of the developing world's population while half of the population in industrialized countries use the traditional medicine for treatment of human and animal diseases(Shaikh and Hatcher, 2005). All the respondents in the study area were experienced and knowledgeable people having vast experience of raising livestock and keeping pet animals. Collection of rich data on existing EVPs used for bovine mastitis in rural areas of West Singhbhum district, Jharkhand indicates that livestock owners use the traditional system of veterinary medicine for primary health care system same as stated by (Jain and Srivastava, 2003). Plants constitute major part of EVM so most of the remedies reported in the present study are based on plants. India is endowed with the wealth of medicinal plants. India ranks second in the world after China in export of medicinal plants. Like in other parts of the world, livestock owners have an excellent knowledge of ethno-botany where it has formed the basis for screening plant materials as potential source of medical drugs (Matekaire and Bwakur, 2004).

5.2 Most frequent ITKs for the treatment of mastitis

In the present study use of different plant materials used in different ITKs like powdered root of *Cucurma longa* , seeds of *Brassica campestris*, bulb of *Oryza sativa and Allium sativum*, root of *Zinziber officinale,* seeds of *Foenicum vulgare and Nigella sativa* having similar resuts as described by Dilsad *et al*. (2010). During the present study root of *Glycyrrhiza glabra*, *Nardostachys Jatamansi, Asparagus reacemosus* and leaf of *Ocimum sanctum* used as alternative therapy on the basis of Ethnoveterinary practices (Kolte, 2008). Use of the fruit of *Phyllanthus emblica* against mastitic pathogens was studied by Sharma *et al*. (2010). During clinical mastitis, the inhibitory response of *Azadirachta indica* extract on nitric oxide production by milk leukocytes as indicated by De and Mukherjee, (2009). Antibacterial Activity shown by the leaf of *Centella asiatica* against *Staphylococcus aureus* in bovine mastitis was described by Taemchuay *et al*. (2009). In the present study deals with

leaves of *Lawsonia inermis* depicted maximum antibacterial potential against tested pathogens (Devi *et al*., 2009). In the reported ITKs anti-inflammatory activity of leaves of *Luffa cylindrica* was observed (Khan, 2013). Seeds of *Trachyspermum ammi* and jaggery used by local people of Jharkhand revealed results similar to Bilal *et al*. (2009), Mohanta *et al*. (2007) prepared the aqueous and organic solvent extracts of the same plant and screened for antimicrobial properties. Gopinath *et al*. (2011) investigated antibacterial activity of *Punica granatum* L. against pathogens causing bovine mastitis. The plant *Tabernaemontana divaricata* is having great antimicrobial properties against mastitis pathogens. Results of the present study regarding antibacterial activity of *Datura stramonium* are in conformity to earlier finding of Uzun *et al*. (2004), who found antibacterial activity against both *S. aureus* and *E. coli*.

5.3 Most frequently used plant for the treatment of mastitis based on FL% value

A number of EVPs were reported by the respondents for prevention and treatment of mastitis. In the present study most frequently used plant for the treatment of mastitis in bovine, bubaline and small ruminants were bark of *Listea monopatela, Bombex Ceiba* and *Butea monosperma*, fruit of *Piper longum* and *Terminalia bellerica* and root of *Glycyrrhiza glabra.* Ethanolic extract of *Litsea monopetala* demonstrated the presence significant antimicrobial, anti-inflammatory and anti-atherothrombosis activities (Ahmmad *et al*., 2012). *Glycyrrhiza glabra* extracts showed significant antibacterial activity against gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative (*E. coli* and *Pseudomonas aeruginosa*) bacteria (Nitalikar, 2010). A great antimicrobial activity has shown by piper fruits against bacterial pathogens (Khan and Siddiqui, 2007). Alam, (2011) postulated that crude methanolic extract of the fruits of *Terminalia bellerica.* Roxb along with its various organic fractions elicited both *in vitro* and *in vivo* antioxidant activity as well as antibacterial activity. *Butea monosperma* is useful as anti-inflammatory activity and helps in reduction of dermal wounds (Sumitra, 2005). *Bombex Ceiba* plant has been found to possess strong anti-inflammatory, antibacterial, antiviral, analgesic, antioxidant as well as fibrinolysis enhancing activities (Dar, 2005).

5.4 Ethno-veterinary remedies: Preparation, dosage and administration

Many of the ingredients used for treatment of mastitis by the farmers are found in every household used to cure ailments, most of the times the plants that are indigenous to the area are collected by the EVM users and practitioners themselves or purchased from the nearby herbal shops (Farooq *et al*., 2008). For preparation of drug, parts of the plant that were mostly utilized by the respondents were roots, bark, wood, leaves, stems, flowers, fruits, resin, latex, grains, buds, bulbs and seeds as reported in many studies (Giday *et al*., 2003). The common method of extraction of drug was through grinding, soaking, brewing or boiling different parts of plants or whole plant and Jaggery, butter, milk fat and wheat flour were used as vehicles (Abbas *et al*., 2002). The respondents reported that they administer the remedies to the animals mostly orally as decoctions, liquid in that the plants have been steeped, through fumigation, topical application or as external washes (Tabuti *et al*., 2003). Farmers do not have exact knowledge about the doses they were of the opinion that dose of the drug depends on size of animal or availability of plants used for treatment. Non-standardized dosages are subjected to criticism by the veterinarians (Longuefosse and Nossin, 1996). In EVPs the duration for that treatment for a particular disease is applied is highly variable and depends largely on the owner of the animal. The clinical improvement of affected animals usually is considered the end of that disease condition (Jabbar *et al*., 2006a).

5.5 Present status of Ethno-Veterinary Medicine in the study area

West singhbhum district have network of veterinary hospitals in urban areas but majority of the respondents were not satisfied with the government-run veterinary services. Travelling veterinary services are available but rare visits made by the veterinary officers/assistants in this regard concluded that shrinking financial resources of the governments make them unable to provide better quality animal health services. Because of low cost, no-side effects, more effective than allopathic treatment and inherited this knowledge from their ancestors farmers prefer to use EVM (Padmakumar, 1997). So certain advantages of EVM over allopathic treatment make it first preference of the farmers to be used for the treatment of animal diseases (Mathias, 2004). Majority of the farmers were not able to tell exact time frame when they started using

the traditional practices. Most of them were between the ages of 47-64 years. This shows that traditional practices have been used for a long time. All of them agreed that if the animals are provided with the traditional treatment in the early stage of disease than there are more chances of recovery of the animal.

In the present study, it was reported that most of the times farmers doing treatment by themselves, so seeking advice from the elders. The results are in agreement with the study conducted by Padmakumar (1997) that most of the remedies are advised by the elders. People prefer to consult the traditional healer because of their cooperation, empathetic attitude and active listening they attract more clientele as compared with modern allopathic practitioners, they are highly respected in the society and are said to be experienced in their work (Shaikh and Hacher, 2005). Moreover, another very strong reason for not consulting a veterinary doctor is the general satisfaction with the performance of the local healers who prove to be quite efficient in offering treatment (Misra and Kumar, 2004). In the present study most of the traditional/road-side healers interviewed were between the ages of 47-64 years. Their low educational status causes no hindrance in their profession even in this modern time. All of them were well experienced having experience of more than 40 years. It shows their dedication to the profession and it's since long that domestic animals are benefiting from this reservoir of knowledge. They acquired knowledge from father–to-son communication or from some senior practitioners as stated in different studies (Zuberi, 1997; Mathias and McCorkle, 2004). In the present study area the healer's deals with specific animal species only and this is not in agreement with the studies concluded that livestock healers often attend to both people and animals (Mathias, 2004). A strong perception exists among the animal owners that evil-eye is the cause of ailment in their animals especially if their animals are high producers or good in performance. It strengthens the supernatural aspects or medico-religious practices in the society. These are practiced by the saints/peer (Religious men). They have special position in the society because of the power that they pretend to possess or acquired. People use to combine the religious and other medicinal practices to cure their animals. Same type of concepts exist in other parts of the world as well (Kambewa *et al*., 1997). To have an insight on ethno-veterinary status in the study area the veterinary officers and veterinary assistants were interviewed. They

were of the view that EVM is always first choice of the small-scale farmers because of limited access to the conventional drugs and veterinary services either due to their non-availability or high costs (Pieroni *et al*., 2004; Jabbar *et al*., 2006a). They were of the opinion that farmers prefer the EVM because allopathic treatment is said to be associated with side-effects. They also reported that the difficulties in the collection of herbs, preparation of drugs and their administration now a days are found to be important constraints in the utilization of ethno-veterinary medicine same as stated by Padmakumar (1997). Many of the plants narrated in the present study have been reported in literature having chemical properties that justify their use. Many plants documented in the present study are reported in the literature but with different veterinary uses. The results reported in the present study are preliminary findings. Many of the natural products used in these practices are reported in the literature to be environmentally sound and with few hazards. So, the problems of pollution and residues in food (Hammond *et al*., 1997; Nfi *et al*., 2001) can be solved by finding new alternatives in the form of natural products. The efficacy of the traditional practices is still open to question so further research is needed to confirm the claims made by the farmers and the traditional healers.

5.6 Identification of Mastitis Pathogens

Coagulase Positive *Staphylococcus aureus* known to be a major cause of bovine mastitis as reported by (Barkema *et al*., 2006; Juhasz-Kaszanyitzky *et al*., 2007). Presence of the coagulase enzyme is an important phenotypic determinant of Coagulase Positive *Staphylococcus aureus* (NMC, 1999; Pyorala and Taponen 2009). Coagulase Negative Staphylococcal species (CNS) and streptococcal species were also major sources of infection. CNS species is to be the most important common causative agent of mastitis similar those reported by (Tenhagen *et al*., 2006). *Streptococcus agalactiae* is known to be one of the major contagious pathogens causing bovine mastitis (Barkema *et al*., 2009) and the identification of *Streptococcus agalactiae* demonstrated in this study was based on several publications (Facklam, 2002). The importance of *E. coli* mastitis is due to its increasing incidence and the severe symptoms like milk drop and fever as observed by Schukken *et al*. (1989).

5.7 Susceptibility of Mastitis Pathogens to herbal extracts/ biodynamic agents and standard antibiotics

Streptococcus agalactiae species identified were overall found to be highly susceptible to the antibiotic Ciprofloxacin used in this study. This finding was inconsistent with what (Joachim *et al*., 2009) shown in previous publications. In this study antimicrobial activity exhibited by methanolic extract of *Glycyrrhiza glabra* roots against *St. agalactiae* similar to the observation obtained by (Gupta *et al*., 2008) against both Gram-positive and Gram- negative bacteria as well as mycobacteria. In the present study *Listea monopetala* exhibiting maximum zone of inhibition against *St. agalactiae* consistent with the result with (Ahmmad *et al*., 2012) showed varying degrees of antimicrobial activities against 4 Gram positive and 7 Gram negative pathogenic bacteria and 7 fungi using ciprofloxacin and fluconazole respectively as standards. In this study the attempt was made to evaluate the antimicrobial activity of various solvent extracts of fruit of *Piper longum* L. against different gram positive and gram negative bacteria by using disk diffusion method, results were similar as described by (Scherrer, 1971) that gram-positive bacteria were more susceptible to *Piper longum* species because they have only outer peptidoglycan layer which is not an effective barrier. Methanolic extract of *Terminalia bellerica* showed great antimicrobial activities similar to results indicated by (Elizabeth, 2005) that crude and methanol extracts of *Terminalia bellerica* dry fruit possessed broad spectrum antimicrobial activity.

5.8 Therapaeutic Trial

Streptococcus agalactiae is a highly contagious, obligate bacterium of the bovine mammary gland. This bacterium can survive a very short time in the environment, but it can persist indefinitely within the mammary gland as an obligate pathogen of the udder (Keffe, 1997). *St. agalactiae* is a highly contagious obligate parasite of the bovine mammary gland (McDonald, 1977). The costs associated with the use of cows or goats to assess the *in vivo* efficacy of new antibacterial compounds constitute a major drawback. Therefore, the present study was envisaged to characterize the antibacterial potential of methanolic extracts of *Glycyrrhiza glabra, Listea monopetala* and *Piper longum* in mouse model of intramammary infection.

The trial was carried out in the Animal shed, Division of Medicine, Indian Veterinary Research Institute, Izatnagar during the year 2013-2014 in lactating mice challenged with *St. agalactiae* by intramammary route which were compared with lactating healthy mice. The lactating herd of Cattle and Buffalo Farm, Livestock Production and Management (LPM) section, IVRI, Izatnagar, India was screened for mastitis. Milk samples were collected aseptically from the lactating cows after screening for mastitis by California Mastitis Test. *St. agalactiae* isolate which qualified all the cultural and biochemical parameters was chosen for therapeutic trial. Plants extracts/biodynamic agents were chosen for treatment on the basis antibiotic disc sensitivity test and minimum inhibitory concentration test. Mouse mastitis model simulates the ruminant udder in histological aspect to ease the comparative histological and microbiological analysis. Similar to cows, mice have two pairs of mammary glands in the inguinal region, in addition to three pairs in their thoracic body area which are absent in cattle. The glands of both the species are functionally and anatomically independent from each other. In addition, each mammary gland has only one teat opening and one primary duct. As in cows, the mouse mastitis model also provides the unique pathogen growth environment of milk and allows interaction of the organism with the host cells and immune components, besides offering physical factors such as suckling. The observations concerning bacterial counts, neutrophil numbers and histological changes in mice are similar to those in cows (Chandler, 1970b). In addition to that, small size of mouse mammary gland allows it to be completely removed and fixed intact (Notebaert and Meyer, 2006). Chandler (1970a) was probably first to describe the method of intramammary inoculation into lactating mammary glands. Mouse model mastitis has many advantages over cattle which include cost, repeatability, opportunities for microbiological or histological examination. Mice mastitis model has been experimentally used by various workers for studies on pathogenesis of various infectious agents and their products (Bramley *et al.,* 1989). Only a few murine studies have studied for *St. agalactiae* as a mastitis pathogen (Notebaert & Meyer, 2006; Trigo *et al*., 2009). For an obligate intramammary pathogen like *St. agalactiae*, the bovine udder is recognized as the only reasonable source of the organism in the milk. Consequently, isolates in the bulk tank are usually assumed to have come from the udder. In addition, localization of bacteria in epithelial cells, neutrophils and macrophages in

the mouse mammary gland has been reported (Brouillette *et al*., 2004). Mice mastitis model has also been used by various workers for studying therapeutic actions of various antibacterial agents (Craven and Anderson, 1982).

5.8.1 Determination of colony forming unit (cfu) of challenge strain

St. agalactiae isolate was selected as experimental pathogenic strain for the induction of mastitis in mouse model and the therapeutic trial thereafter. An inoculum of 10^8 CFU of *St. agalactiae* (Trigo *et al*., 2009) isolate per gland caused an important level of intramammary infection. Administration of plant extracts along with honey reduced the number of CFU/g of gland in the experimental mice.

5.8.2 Challenge of mice by *St. agalactiae* isolate

The experimental trial was conducted in 7 days post litter lactating mice using right- 4 (R-4) and left-4 (L-4) inguinal mammary glands. Forty-two 7 days post litter lactating mice were divided into 7 groups comprising of 6 animals each. Group I (healthy control), Group II (infected untreated control), Group III (*Glycyrrhiza glabra* + Honey treated), Group IV (*Piper longum* + Honey treated), Group V (*Listea monopetala* + Honey treated), Group VI (Combination of *Glycyrrhiza glabra*, *Piper longum*, *Listea monopetala* and honey treated) and Group VII (Ciprofloxacin) animals were used for the trial. Except Group I animals, all the mice were challenged with 18 h incubated broth culture of *St. agalactiae* isolate at the dose rate of 0.1 ml containing approximately 10^8 cfu/ ml through R-4 and L-4 inguinal mammary glands. The cases of mastitis were screened by visual and microbiological examinations were graded using clinical examination score card (Sridevi, 2005).

5.8.3 Clinical score card for the evaluation of clinical symptoms before and post therapy

5.8.3.1 Pre-challenge scores

All the animals in all the groups (I, II, III, IV, V, VI and VII) were found healthy and normal in demeanor with no gross pathological abnormalities like swelling, discolouration or exudation of mammary glands as per Evans postulates (Evans, 1976).

5.8.3.2 Score card evaluation at 24 h post-challenge

No gross destruction of tissue indicating necrosis was seen in all challenged animals only mild swelling present in few animals of challenged group simulated with findings of Chandler (1970b). In a previous study by Trigo *et al*. (2009) that within 24 h of infection, *Streptococcus agalactiae* had multiplied and spread throughout the mammary tissue, as shown by histological analysis of the mammary glands. These results suggested a lag phase in the ability of *Streptococcus agalactiae* to adapt and grow in the mammary gland.

5.8.3.3 Score card evaluation at 48 h post-challenge

Only mild swelling and slight reddish discoloration was observed in mammary gland of Group II challenged animals. Mild swellings were also observed in 4 mice from Group III animals, 3 mice from Group IV and V and 2 mice from Group VI and VII animals. These showed animals were resisting the infection effectively. It has been suggested that phagocytic potential affect the pathogenesis of the disease, these findings were in complete agreement with observation by Smith and Chadler (1978).

5.8.3.4 Score card evaluation at 96 h post-challenge

In Groups III, IV, V, VI and VII the general demeanor was almost normal and only mild swelling were observed in some animals. It appears that the biodynamic agents/herbal extracts exerted some exfiltrative influence during inflammatory reaction and ameliorated the infiltration by PMNs. Lakshmi *et al*. (2011) showed that *Glycyrrhiza glabra* had the potential of reducing inflammation and simultaneously possessed antibacterial activity against Gram positive and Gram negative organisms (Sultana *et al*., 2010). The antiinflammmatory activities of *Piper longum* was reported by Gupta (2003). Also studies conducted by (Ahmmad *et al*., 2012) showed that some active principles in *Listea monopetala* were responsible for amelioration of inflammation. In contrast, the uninfected mice in Group I was clinically normal with no gross pathological alterations.

5.8.3.5 Score card evaluation at 144 h post-challenge

The mice in Group II were having moderate inflammatory reaction in mammary glands and depression in some of the mice. This study has shown that *Streptococcus agalactiae* can

adhere to and be internalized into host cells (Rubens *et al*., 1992), which could explain why subclinical infections with *Streptococcus agalactiae* tend to become chronic. This was in marked contrast to the observations made in Group I animals, which revealed normal general demeanor.

In Groups III, IV, V and VI the animals were little dull in behavior, the possible reason for such a behavior could be due to immunomodulatory effects of herbal extracts/biodynamic agents. In GroupVII mice receiving Ciprofloxacin revealed only mild swelling and the most important finding was only mild infiltration by neutrophils as opposed to positive control. Strikingly similar results were obtained by Brouillette and Malouin (2005) which proved that, in a dose dependent manner, antibiotic treatment ultimately reduced the inflammation in terms of PMN infiltration by diminishing the bacterial burden in mammary glands.

5.8.4 Differential leukocyte count (DLC)

Before challenging the mice with *St. agalactiae*, lymphocytes outnumbered neutrophils. However in response to the challenge the reverse trend was observed at 48 h, 96 h and 144 h PC. Such significant difference were not noticed in the numbers of monocytes, eosinophils and basophils. This may has been because their numbers are very low in normal mice and even if their number varies after infection they are too low to be detected significantly. Also, in the present study the significant alteration in the relative number of neutrophils and lymphocytes was noticed only in the positive control group. Whereas in Groups III, IV, V, VI and VII mice no significant alterations was observed. The most obvious reason appeared to be acombination of anti-inflammatory and antibacterial effects of biodynamic agents (Gupta *et al*., 2008; Ahmmad *et al*., 2012; Gupta, 2003). In positive control group significant ($p<0.05$) increase in number of neutrophils from a mean value of 13.33% to 50.33% at 48 h and decrease in lymphocyte numbers from a mean value of 78.83% to 41.50% was observed. Brouillette and Malouin (2005) reported similar changes in DLC in mice induced with *Staphylococcus aureus*.

In case of Groups III, IV, V and VI animals the neutrophil number did not increase significantly ($p<0.05$) which could be because of therapeutic effects of these herbal extracts/ biodynamic agents. In healthy control Group I animals, insignificant changes in the relative number of neutrophils and lymphocytes were observed.

5.8.5 C-reactive protein (CRP)

The semi-qualitative analysis of C reactive protein was performed in the blood serum of experimental animals. The results of qualitative analysis ranged from negative (-) to positive (+). The C- reactive protein in animals of Group II *i.e.* infected control ranged from negative (-) at 0 h to positive (+) at 48 h PC. The results are in direct agreement with the findings of (Sarikaputi *et al*., 1991; Kilpatrick and Volanakis, 1991) who established that C-reactive protein plays a major role in destroying infectious agents, minimizing tissue damage facilitating tissue repair and helps in tissue regeneration. Animals from Group III, IV, V and VI revealed presence of CRP in their blood serum at 24 h and 48 h. Thereafter, the level of CRP diminished drastically at 96 h PC. This study was similar to the observation of Liu (2008) who reported CRP is mainly used as a clinical indicator of acute infections and in response to treatment helps to assess the inflammatory status in chronic diseases. There could also be a part played by herbal extracts/biodynamic agents and antibiotics in Group III, IV, V, VI and VII where the anti-inflammatory potential of these agents might have an ameliorating effect on inflammation and counteracting the observations observed in Group II animals. The serum samples of Group I animals were CRP negative during throughout the study at 0 h, 24 h, 48 h and 96 h PC.

5.8.6 Interleukin-12 p40 (IL 12 p40)

Mouse IL-12p40 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Mouse IL-12 P40 in serum. The concentration of IL-12 P40 was higher for Group II animals revealed increase in the level of IL-12p40 after infection required for neutrophil recruitment and is a mediator between innate and acquired immunity similar observation reported by Hornef *et al*. (2002). In Groups III, IV, V, VI and VII animals at there was increase in concentration of IL-12p40 at 96 h from 0 h value but the level was not to the extent as observed in Group II animals. It could be due to immunomodulatory effects of the biodynamic agents before challenging with *St. agalactiae*. Group I animals showing not much alteration in concentration of IL-12p40 at 96 h as compared to 0 h value as observed by (Trinchieri, 1995).

5.9 Histopathological examination

Mice of all the 7 groups were sacrificed at 144 h post challenge for histopathological examination.

In general, Group I healthy uninfected negative control mice had normal lactating alveoli with eosinophilic granular contents and no pathological alterations. These observations were in accordance with previous report by Chandler (1970b).

The Group II mice revealed severe inflammatory changes, necrosis of mammary glands characterized by cellular infiltration with distinct neutrophils and disruption of normal architecture of the gland similar to findings of (Trigo *et al*., 2009).

In Groups III, IV, V and VI animals receiving herbal extracts or biodynamic agents the inflammatory reaction was mild in severity and the cellular architecture was only partially lost. Fewer necrotic foci and a mild fibrocellular reaction were observed. This effect was possibly because of the biodynamic agents acting on inflammatory cascade parameters.

In Group VII animals the mammary gland displayed a relatively less pathological contour than positive control. The inflammatory reaction was mild.(Brouillette and Malouin, 2005) observed that in mouse mastitis model treatment with antibiotic Cephapirin @ 10-25 mg/kg body weight reduced the PMN infiltration by 71-85%.

5.10 Demonstration of bacteria in mammary gland tissue sections of infected untreated Positive control group animals

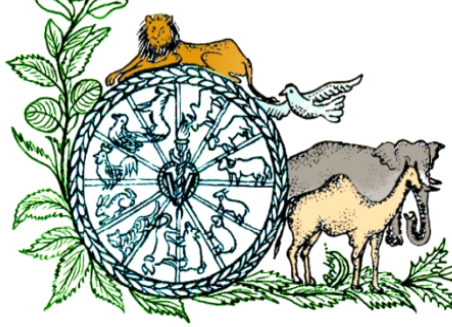
On staining with Gram's and Giemsa stain, Gram positive cocci indistinguishable from *Streptococcus agalactiae* were demonstrated in tissue in agreement with the postulates laid down by Koch (Koch, 1892).

5.11 Enumeration of bacteria in mammary gland tissue triturate

L-4 mammary gland triturates of mice from each group were inoculated on nutrient agar and incubated at 37°C for 16-18 h and counted with the help of colony counter. No bacteria were isolated from healthy negative control mice. In Group II, the mean viable count

of bacteria was 6.40×10^6 which followed the same trend as Chandler (1970a). In Groups III, IV, V and VI animals the mean viable counts were lower than those found in positive control. In Group VII animals the viable counts were lowest among the infected groups which were attributed to the bactericidal effects of antibiotic ciprofloxacin.

Summary and Conclusions



6 SUMMARY AND CONCLUSIONS

Ethno-veterinary medicine (EVM) is a system that is based on traditional knowledge, folk beliefs, skills, methods and practices used for maintaining health of animals and curing diseases. Like all other traditional knowledge systems, traditional veterinary medicine knowledge is handed down orally from generation to generation and it may disappear because of rapid socioeconomic, environmental, technological changes and as a result of loss of cultural heritage. So documentation is the primary need in case of systematic studies to be conducted for conservation of this valuable asset.

In the present study, three hundred and twenty randomly selected livestock raisers in twelve villages of two sub division in West Singhbhum district of Jharkhand were interviewed. Number of respondents selected from these areas was based on the population of the particular village. An initial participatory Rural Appraisal (PRA) was conducted in the rural areas of West Singhbum district of Jharkhand. This intended to provide primary data on size of holdings and species of animals owned by each producer as a basis for selecting respondents for the data collection. For collection of information on ethno-veterinary practices in the rural areas, three hundred and twenty key respondents (livestock raisers), thirty traditional/road-side healers and eight Veterinary officers / Veterinary assistants) from outskirts were randomly selected and interviewed. Interviews, field visits and focused group discussions were used as the tools of Participatory Rural Appraisal (PRA) and validated in field by QuIK and in labroratory by in vitro and a mouse model of intramammary infection has been characterized for in vivo testing of prophylactic efficacy of herbal extracts based on the information collected.

Information was collected using pre-designed questionnaires and open-ended interviews. Arrangements for photographs and audio-visual records were made whenever considered necessary. A total of 40 plant species were identified for the treatment of bovine mastitis and10 ITKs based on plant usage, combination of plant(s) or other material(s), chemicals/ dairy or animal products/organic materials and other practices were documented in cattle and buffaloes for the treatment of bovine mastitis. In the present study, 40 plant species and 29 families were reported by the respondents. Medicinal plants having more than 70% FL values were *Listea monopetala* (95%) followed by *Piper longum* (85%), *Glycyrrhiza glabra* (80%), *Terminalia bellerica (80%). Bombax ceiba (75%), Butea monosperma* (75%). These selected herbal plants were further used as therapeutic trial in mice.

There was a wide variation in dosage and no standard procedure existed for preparation and administration of remedies. People have easy access to modern veterinary facilities but still traditional remedies and traditional healers are their first choice. Ethno-veterinary users (animal owners) and ethno-veterinary practitioners have a strong perception over the effectiveness of these remedies. Moreover they reported that allopathic treatment is expensive and sometimes associated with side-effects. Veterinary officers and veterinary assistants agreed upon the extensive use of EVPs in the study area.

The selected six medicinal plants are the source of the secondary metabolites i.e., alkaloids, flavonoids, tannins, glycosides and steroids. Medicinal plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases.

The trial was carried out in the Animal shed, Division of Medicine, Indian Veterinary Research Institute, Izatnagar during the year 2013-2014 in lactating mice challenged with *St.*

agalactiae by intramammary route, which were compared with lactating healthy mice. Milk samples were collected aseptically from the lactating cows of Cattle and Buffalo Farm, IVRI after screening for mastitis by CMT. Pathogenic *St. agalactiae* isolate was chosen as a challenge organism. The herbal extracts, their combinations and antibiotics revealed best result against isolate of *St. agalactiae*. The experimental trial was conducted in 7 days post litter lactating mice using right- 4 (R-4) and left (L-4) inguinal mammary glands. Forty-two 7 days post litter lactating mice were divided into 7 groups comprising of 6 animals each. Group I (healthy control), Group II (infected untreated control), Group III (*Glycyrrhiza glabra* + honey treated), Group IV (*Piper longum* + honey treated), Group V (*Listea monopetala* + honey treated), Group VI (combination of *Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* + honey treated) and Group VII (Ciprofloxacin treated) animals were used for the experiment. Except Group I animals, all the mice were challenged with 18 h incubated broth culture of *St. agalactiae* isolate at the dose rate of 0.1 ml containing approximately 10^8 cfu/ ml through R-4 and L-4 inguinal mammary glands. The cases of mastitis were screened for disease status and graded using clinical examination score card.

The total clinical scores remained 0.00±0.00 at 0 h, 24, 48 h, 96 h and 144 h of observational period in Group I mice. In Group II infected untreated control animals the total clinical scores ranged from 0.00±0.00 to 3.00±0.00 at 0 h, 24, 48 h, 96 h and 144 h. The total clinical scores increased ($P<0.05$) significantly to an extent of 8.33% at 96 h and 144 h PC as compared to 24 h value in Group II animals. The total scores decreased($P<0.05$) significantly to an extent of 22.22% in *Glycyrrhiza glabra* + honey treated Group III animals at 96 h and 144 h PC as compared to 24 h value. In Group IV *Piper longum*+ honey treated mice, the scores decreased ($P<0.05$) significantly to an extent of 25.00% at 96 h and 144 h PC as compared to 24 h value. While, in *Listea monopetala* + honey treated Group V animals the total clinical scores decreased ($P<0.05$) significantly to an extent of 33.33% at 96 h and 144 h PC as compared to 24 h value. In *Glycyrrhiza glabra*+ *Piper longum*+ *Listea monopetala* + honey treated Group VI animals the total clinical scores decreased ($P<0.05$) significantly to an extent of 44.44% at 96 h and 144 h PC as compared to 24 h value. The antibiotic treated Group VII revealed best response to treatment. In this group the total clinical

scores decreased ($P<0.05$) significantly to an extent of 55.55% as compared to infected Group II animals at 96 h and 144 h PC as compared to 24 h value.

The following changes were recorded in DLC of experimental mice during challenge and infection. In healthy mouse the numbers of lymphocyte exceeded the count of neutrophil. In the present study, relative number of lymphocytes decreased significantly ($P<0.05$) in all the challenged groups (Group III, Group IV, Group V, Group VI and Group VII) as compared to healthy group at 48 h, 96 h and 144 h PC. Significantly ($P<0.05$) higher neutrophil levels were recorded in all the infected groups as compared to healthy group (Group I) at 48 h, 96 h and 144 h PC. Group II animals recorded an increase of 69.54%, 69.13% and 71.52% in the relative percentage of neutrophils at 48 h, 96 h and 144 h PC respectively as compared to 0 h value. The level of neutrophils remained same in the Group I healthy animals throughout the study.The number of monocytes increased ($P<0.05$) significantly in all the extract treated groups (Group III, Group IV, Group V, Group VI) as compared to the healthy Group I animals at 48 h, 96 h and 144 h PC. However, in antibiotic treated Group VII the number of monocytes increased significantly ($P<0.05$) at 96 h PC respectively.

In the present study, semi-qualitative analysis of C-reactive protein was performed in the serum of experimental animals. The CRP in positive control animals was negative at 0 h. In CRP study 6 animals from Group II, 2 animals from Group III and Group IV, 1 animal from Group V, VI and VII was positive during 24 h and 48 h PC. The level of CRP reduced drastically at 96 h PC. Concentration of IL-12p40 was higher in Group II animals followed by other treated groups at 96 hr. Group I animals showed not much alteration in concentration of IL-12p40 at 0 hr and 96 hr. Increase in concentration of IL-12p40 of groups IV, III, V, VI and VII at 96 h revealed infection is established and some inflammatory reaction was going on.

Animals of all groups were sacrificed at 144 h PC while the infected untreated controls were sacrificed for histopathological examination of the mammary glands at 0 h, 24 h, 48 h and 96 h. Tissue sections of mammary gland of Group I healthy lactating mice revealed normal healthy lactating alveoli with eosinophilic contents and no signs of inflammation. Distribution of red collagen fibers was restricted to teat region only and interlobular tissue was visible as thin

septa between lobules. However, in the infected untreated mice of Group II revealed inflammation of alveoli containing reduced quantity of eosinophilic granular material and thickened inter-acinar septa as compared to Group I healthy mice. Multiple focal micro-abscesses predominated with neutophils and fibrinous exudates with mild fibrosis were evident in the mammary parenchyma. In the *Glycyrrhiza glabra* + honey treated Group III, tissue sections of mammary gland revealed distended and non- distended alveoli with mild inflammatory reaction as compared to the infected untreated control. Fewer necrotic foci with lesser fibrocellular reaction were noticed in the mammary gland tissue sections as compared to the infected untreated control. In *Piper longum*+ honey treated Group IV animals tissue sections of mammary gland revealed comparatively reduced inflammatory and collagenous fibrocellular reaction. Whilst, tissue sections of mammary gland of *Listea monopetala* + honey treated Group V animals revealed both non-distended and distended alveoli with scanty inflammatory exudate of mononuclear cells but the tissue architecture appeared almost normal. In *Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* and honey treated Group VI animals, tissue sections revealed non-distended alveoli with milder inflammatory reaction, moderate mononuclear cell infiltration and fibrosis. Tissue sections of antibiotic treated Group VII appeared almost normal with mild inflammatory reaction in comparison to any other treated group.

In the inflammed mammary gland tissue sections stained by Gram's and Geimsa staining techniques, gram positive cocci indistinguishable from *St. agalactiae* were observed. No viable organism could be isolated from the mammary glands of healthy control. In the infected untreated Group II animals, high mean bacterial count of 6.40×10^6 was obtained at 144 h PC. Mean viable count in Group III mice treated with *Glycyrrhiza glabra* + honey was 3.80×10^4. In Group IV animals treated with *Piper longum* + honey mean viable count of $3.85 \times 10^4$was observed. In Group V animals treated with *Listea monopetala* + honey revealed mean viable count of 3.70×10^4. Mean viable count in Group VI animals treated with *Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* and honey mean viable count of 3.60×10^4 was observed. In antibiotic treated Group VII animals a low mean viable count of 3.00×10^3 was obtained.

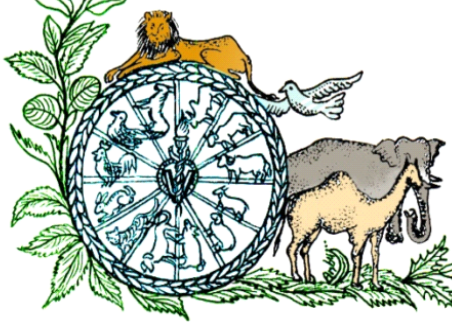
This is the first planned study on documentation of EVPs used for bovine mastitis in livestock in rural areas of West Singhbhum district of Jharkhand. The study revealed diversity of ethno-veterinary practices used for livestock.

Following conclusions have been draws based on the present study :

1. Phytotherapy is the core of these traditional practices. Many of the plants documented in the present study have been reported in literature having chemical properties that justify their use. Many plants documented in the present study are reported in the literature but with different veterinary uses.

2. In vivo efficacy was maximum for antibiotic treated Group VII (Ciprofloxacin) > Combination of herb (*Glycyrrhiza glabra* + *Piper longum* + *Listea monopetala* and honey treated group) > Group V (*Listea monopetala* + honey) > Group III *(Glycyrrhiza glabra* + honey) > Group IV (*Piper longum* + honey).

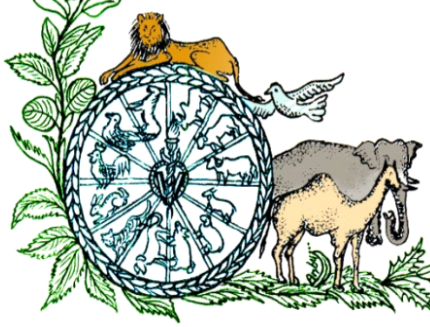
Mini Abstract



7 MINI ABSTRACT

Ethno veterinary information is in danger of extinction because it is less systematic, less formalized and advancement of modern veterinary practices. Both types have their strengths and limitations and in some instances, they complement each other. Thus it has become imperative to collect and document these practices and to assess their validity was felt as an urgent need. With this scenario, A study on "Collection, documentation and validation of some ethnic biodynamic agents used by the tribal communities of West Singhbhum District of Jharkhand against bovine mastitis" was taken up during 2013-2014. A semi structured interview schedule was used for data collection, on the basis of that 40 herbal products and 10 Indigenous traditional knowledge (ITKs) were recorded. These herbal products and ITKs were subjected to validation test through Quantification of indigenous knowledge (QuIK) method. The data analysis for 40 herbal products was done by calculating Fidelity Level (FL %) for a particular ailment. Six herbal products having greater FL% value were further chosen for laboratory validation both in vitro and in vivo validation. After in vitro validation only three (*Glycyrrhiza glabra*, *Piper longum* and *Listea monopetala*) was giving the better result against *Streptococcus agalactiae* (*St. agalactiae*). These products were further subjected to in vivo validation in lactating mice by creating mouse model mastitis by organism *St. agalactiae*. The therapeutic potential of herbal products and antibiotics were studied in mice and evaluated through clinical score card, differential leukocyte counts, acute phase reaction, pro-inflammatory cytokines and histopathological changes. The total clinical scores were significantly ($P<0.05$) higher in infected mice of Group II as compared to healthy control group and challenged groups (III, IV, V, VI and VII). The relative percentage of neutrophils increased significantly ($P<0.05$) at 48 h, 96 h and 144 h post challenge in infected positive control group mice. Qualitative analysis for C- reactive protein revealed that all the animals of infected positive control group, two animals from group III and IV. One animal from group V, VI and VII showed positive result at 24 h and 48 h PC. IL12 p40 concentration in serum was higher for infected positive control group with respect to other challenged groups (III, IV, V, VI and VII). Regarding histopathological findings severe necrosis, loss of cellular architecture and disruption of normal alveoli were observed in infected positive control group mice compared to normal healthy control group and infected groups (III, IV, V, VI and VII). Mammary gland tissue sections revealed the presence of gram positive cocci indistinguishable from *St. agalactiae* on Gram's and Giemsa staining. Bacteriological examination revealed highest viable counts in group II mice compared to other groups.

लघु सारांश



8 लघु सारांश

वर्ष 2013–14 के दौरान झारखण्ड राज्य के पश्चिमी सिंहभूम जिले में गोवंशीय थनैला रोगोपचार में प्रयुक्त 40 वनस्पति उत्पाद तथा 10 स्वदेशी पारंपारिक ज्ञान (आईटीके) के संदर्भ में अर्द्धसंरचित साक्षात्कार सूची के आधार पर जानकारी जनित कर संग्रहित की गयी। इन वनस्पति तथा आईटी के उत्पादों का स्वदेशी ज्ञान अमापन (क्यू यू आई के) पद्धति से विश्लेषण किया गया। एक विशिष्ट रोग/व्याधि हेतु इन 40 वनस्पति उत्पादों का फिडेलिटी–स्तर प्रतिशत (एफ एल%) गणना द्वारा जिनका एफ एल% मूल्य अधिक था उनका प्रयोगशाला में पात्रे एवं जीवे सत्यापन किया गया। पात्रे सत्यापन के बाद केवल तीन ही वनस्पतियाँ (ग्लाइसिरिज्जा ग्लब्रा, पाइपर लोंगम तथा लिस्टिया मोनोपेटाला) के परिणाम स्ट्रेपटोकोक्कस एगैलेक्शि के विरूद्ध अच्छे पाये गये। इन उत्पादों का आगे दुधारू मादा मूषकों में स्ट्रेप्टोकोक्कस एगेलेक्शि उद्यत थनैला रोग में प्रतिजैविकों के साथ चिकित्सय सामर्थ्य का पात्र मूल्यांकन शयनिक अंक कार्ड, भिन्नक श्वेतकाण गणन, तीव्र चरण प्रतिक्रिया, शोथ सर्मथक साईटोकाइन्ज तथा ऊतक विकृति की बदलाव के आधार पर किया गया। कुल शयनिक अंक समूह 2 के संक्रमित मूषकों में सार्थकतः अधिक तथा स्वस्थ नियंत्रित तथा आव्हानित (समूह 3,4,5,6 एवं 7) की अपेक्षा अधिक पाये गये। आवाहनित संक्रमण उद्यन (पीसी) के 48, 96 एवं 144 घंटे पश्चात् सकारात्मक नियंत्रण समूह के सापेक्ष उदासीन रंजकाणुओं में सार्थक वृद्धि पायी गयी। पी.सी. के 24 तथा 48 घंटों पर सी–रिएक्टिव प्रोटीन के गुणात्मक विश्लेषण से समूह दो के सभी, समूह तीन एवं चार के प्रत्येक दो मूषक तथा समूह 5,6 एवं 7 में प्रत्येक एक पशु सकारात्मक पाया गया। आई.एल.12, पी–40 का सीरम सान्द्रण अन्य पी.सी. समूह (3,4,5,6 एवं 7) की अपेक्षा समूह–2 में अधिक पाया गया। ऊतक विकृति कि परिणामों में गंभीर परिगलन, कोशिकिय संरचना की क्षति, समूह 2 में पाये गये सामान्य दुग्ध जनन ऊतकों के विघटन की अपेक्षा यह परिवर्त सामान्य स्वस्थ नियंत्रण समूह तथा संक्रमित समूहो (3,4,5,6 एवं 7) के मूषकों में कम पाये गये। स्तन ग्रन्थि ऊतकों में ग्राम तथा जिसरसा रंजन पर जीवाणु पाये गये। जीवाणु परीक्षण से समूह दो के मूषकों में सर्वोच्च जीवित गणना परिलक्षित हुई।

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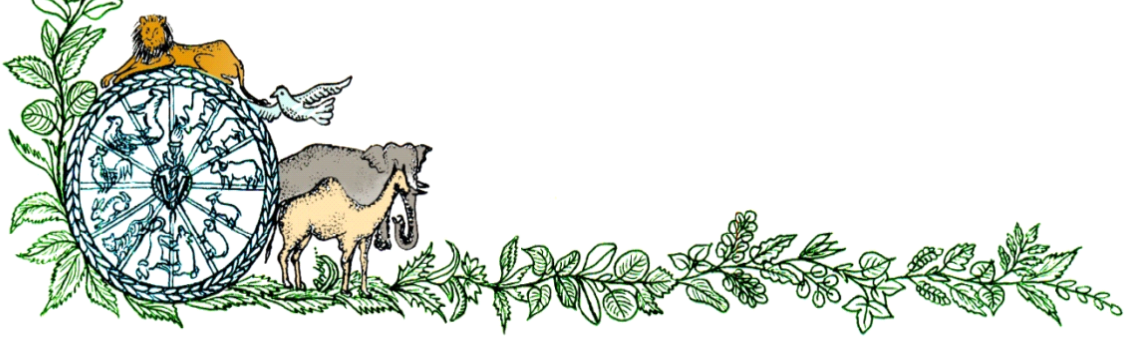

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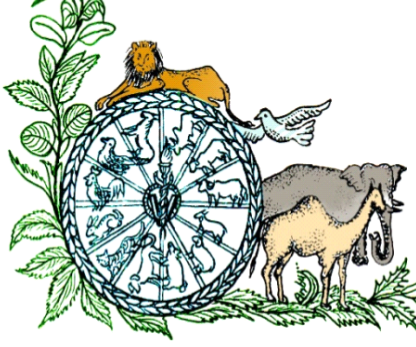
Appendix



APPENDIX-VII

CMT Reagent

Bromocresol purple	5 mg
Sodium Hydroxide (NaOH)	15 gm
Teepol (A.G.)	15 ml
Distilled water	1000 ml

Mix well and store at room temperature.

Normal Saline Solution

Sodium Chloride (NaCl)	9 gm
Distilled Water	1000 ml

Phosphate Buffer Saline

Solution I

Sodium chloride (NaCl)	8 gm
Potassium chloride (KCl)	0.2 gm
Distilled water	1000 ml

Solution II

Disodium hydrogen orthophosphate ($Na_2 H PO_4$)	1.44 gm
Potassium dihydrogen orthophosphate ($K H_2 PO_4$)	0.24 gm
Distilled water	1000 ml

Take solution II in large volume and bring down pH by adding solution I till the pH is 7.4. Sterilize and refrigerate.

Bovine Blood Agar (5%)

Nutrient Agar	6.8 gm
Distilled water	200 ml

Dissolve and autoclave at 15 lbs pressure (121°C) for 15 minutes. Before pouring in plate add 10 ml of sterile bovine blood.

Mannitol Salt Agar

Mannitol salt agar	22 gm
Distilled water	200 ml

Dissolve and autoclave at 15 lbs pressure (121°C) for 15 minutes.

Baird Parker media

Baird Parker agar powder	13.3 gm
Distilled water	200 ml

Dissolve and autoclave at 15 lbs pressure (121°C) for 15 minutes. Add egg yolk tellurite

Edward media

Edward Medium Base	41.33 gm
Distilled water	1000 ml

Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 115°C for 20 minutes. Cool to 50°C and aseptically add 5 to 7% v/v sterile sheep blood. Mix well and pour into sterile Petri plates.

Muller Hinton broth

Muller Hinton broth	21 gm
Distilled water	1000 ml

Dissolve and autoclave at 15 lbs pressure (121°C) for 15 minutes.

Muller Hinton agar

Muller Hinton Agar	22 gm
Distilled water	1000 ml

Dissolve and autoclave at 15 lbs pressure (121°C) for 15 minutes.

Nutrient agar

Nutrient agar	28 gm
Distilled water	1000 ml

Dissolve and autoclave at 15 lbs pressure (121°C) for 15 minutes.

Eosine Methylene Blue (EMB) agar

EMB Agar	35.96 gm
Distilled water	1000 ml

Dissolve and autoclave at 15 lbs pressure (121°C) for 15 minutes.

Rabbit plasma

Blood was collected from ear vein of two healthy rabbits in anticoagulant (heparin), plasma was separated by centrifugation at 3000 rpm for 5-10 min.

Giemsa stain

Methanol	75 ml
Glycerol	25 ml

0.5 Mc Farland Standard

Sulfuric Acid (1%)	99.5 ml
Barium chloride	0.5 ml

Reagent used for histopathology

Formal saline solution	10 %

Formaldehyde solution was added to normal saline solution at a final concentration of 10% (v/v)

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