

--- Short Communication

## COMPARATIVE EVALUATION OF THE EFFECT OF VARIOUS LECTINS ON SHORT TERM LYMPHOCYTE CULTURE IN CATTLE AND BUFFALO

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### ABSTRACT

The present study compares the effect of various lectins; phytohaemagglutinin (PHA), Pokeweed mitogen (PWM) and Concanavalin-A (Con A) on short term lymphocyte culture and morphology of chromosome in cattle and buffaloes. For the comparative studies, the whole blood culture from 5 cattle and 5 buffaloes in triplicate were set up by adding 3 different types of lectins. Results based on physical observation under microscope, indicate best culture and chromosomal morphology when used pokeweed mitogen for lymphocyte culture in case of cattle and buffaloes. However, PHA added cultures exhibited poor lymphocyte cultures in cattle and buffaloes whereas, ConA added culture also yielded adequate proliferation of lymphocytes and good morphology of chromosomes. Therefore, pokeweed mitogen and Con A lectins can be utilized for lymphocyte culture for karyotyping in cattle and buffaloes.

**Keywords:** Pokeweed mitogen, Concanavalin-A, PHA, Lymphocyte culture

## INTRODUCTION

Most lectins are non-enzymatic in action and non-immune in origin. Lectins occur ubiquitously in nature. They may bind to a soluble carbohydrate or to a carbohydrate moiety that is a part of a glycoprotein or glycolipid. Lectins are proteins which are basically mitogens that trigger mitotic cell division *in vivo* as well as *in vitro*, therefore, are being used in lymphocyte culture for karyotyping of various species worldwide. In other words, a mitogen is a chemical substance that encourages a cell to commence cell division, triggering mitosis. A mitogen is usually some form of a protein. There are various types of mitogens; phytohaemagglutinin (PHA), Pokeweed mitogen (PWM) and Concanavalin-A (Con A) etc. commonly used in lymphocyte culture. The PHA and Concanavalin- A act on T cells only whereas Pokeweed mitogen acts on both T and B-cells.

Phytohaemagglutinin is a lectin found in plants, especially legumes. It is found in the highest concentrations in uncooked red kidney beans and white kidney beans (also known as cannellini), and it is also found in lower quantities in many other types of green beans and other common beans (*Phaseolus vulgaris*), as well as broad beans (*Vicia faba*) such as fava beans. It has a number of physiological effects and is used in medical research. In high doses, it is a toxin. Concanavalin A (ConA) is a lectin (carbohydrate-binding protein) originally extracted from the jack-bean, *Canavalia ensiformis*.

ConA is a plant mitogen, and is known for its ability to stimulate mouse T-cell subsets giving rise to four functionally distinct T cell populations, including

precursors to suppressor T-cell (Dwyer and Johnson, 1981). Pokeweed mitogen is a mitogen derived from *Phytolacca americana*. It functions as a lectin.

Lectins serve many different biological functions in animals, from the regulation of cell adhesion to glycoprotein synthesis and the control of protein levels in the blood. Purified lectins are important in a clinical setting because they are used for blood typing (Nathan and Halina, 2004). Some of the glycolipids and glycoproteins on an individual's red blood cells can be identified by lectins. In neuroscience, the anterograde labelling method is used to trace the path of efferent axons with a type of PHA from the kidney bean (Carlson, 2007). A lectin from bananas inhibits HIV-1 *in vitro* (Swanson et al., 2010).

The present study compares the effect of the aforesaid lectins on lymphocyte culture and morphology of chromosome in cattle and buffaloes so that an appropriate lectin can be utilized for the culture.

## MATERIALS & METHODS

Peripheral blood was collected from phenotypically normal 5 bulls each of cattle and buffaloes in a heparinized vacutainer blood collecting tube. Chromosomal preparations were performed by using standard whole blood culture (Patel, 1999) in readily available RPMI medium (Himedia) supplemented with L-Glutamine, fetal calf serum, Penicillin, Streptomycin and Sodium bicarbonate. Each culture was set in triplicate enabling us to add 1% of each of three lectins; PHA, ConA and Pokeweed mitogen (Sigma) in three samples of each

animal. The blood cultures were incubated at 37°C for 72 hours. To increase the relative frequency of prometaphase chromosomes, Ethidium bromide (Sigma) @10 µg/ml was added and to arrest somatic cell division at metaphase stage, Colchicine (Sigma) @ 2 µg/ml was added to each culture for 3 and 1 h respectively, prior to the harvesting. The cells were separated by centrifugation at 150 g for 5 minutes followed by hypotonic treatment with 0.56% KCl for 20 minutes at 37°C and fixed in 3:1 ratio of methanol and acetic acid glacial. Finally, cell suspension about 20µl was dropped on slides and air dried. Slides were conventionally stained by Giemsa stain for screening under the compound microscope to observe metaphase plates and morphology of chromosomes on each slides prepared from each samples in triplicate.

## RESULTS AND DISCUSSION

Physical observation under the microscope revealed a few metaphase cells with poor morphology of chromosomes, were observed on slides prepared from cell pellet obtained from the culture with PHA for both cattle and buffalo species. However, PHA is very effective for short term lymphocyte culture in humans (Yamamura, 1971; Verma and Babu, 1994). Cattle and Buffalo lymphocyte cultures obtained by using lectin, Concanavalin A (ConA) was good and exhibited sufficient metaphase plates on the slide with good morphology of chromosomes. Many workers used ConA for lymphocyte and for other cell cultures in animals (de Petris, 1975; Gartner, 1975; Nicolae *et al.*, 2009). ConA is not used for lymphocyte culture in human but it was used for the growth of human fetal

intestinal epithelial cells (Weiser, 1972). Cattle and buffalo lymphocyte cultures obtained using pokeweed mitogen exhibited very good number of metaphase plates on slide with excellent morphology. This could be because ConA stimulates only T- lymphocyte whereas PWM stimulates both T and B cells. Hence, pokeweed mitogen is being widely used in cattle and buffalo lymphocyte culture (Patel *et al.*, 2006; Patel *et al.*, 2012). Whereas pokeweed mitogen yielded good lymphocyte culture and morphology of chromosomes in human (Patel, unpublished).

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