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Effect of *Withania somnifera* extract on adipose tissue derived progenitors

A. Mangala Gowri, S. Priya and D. Baskaran

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Herbs play an important role in the traditional systems of medicine and have been used in medical practices since olden days. Herbs have the potential to act as therapeutic agents against various human diseases.The present research is designed based on the concept on the use of herbal adaptogen (*Withania somnifera*) to combat contemporarily increasing stress related disorders.The proliferative and differentiation properties of stem cells treated with herbal extracts have shown promise in diseases such as osteoporosis, neurodegenerative disorders and other tissue degenerative disorders (Bickford *et al.,* 2006). These herbs help to balance cortisol levels which are associated with healthy energy levels and help the body to maintain restful sleep at appropriate times (Kaur *et al.,* 2004). These adaptogens boost the immune system and improves the general well-being and slows down the aging process and revitalizes the entire body by stimulating the production of stem cells (Bhattacharya and Muruganandam, 2003). The aim of the research work is to assess the proliferative potential of Indian adaptogenic herb namely *Withania somnifera* on adipose tissue derived cells from Indian dogs.

Withania somnifera was used in tested concentration in individual and combinations on dog adipose tissue derived cells. Five gram of herbal sample was extracted with 100 ml 80 per cent methanol, vortexed for 40 seconds and incubated at 65°C for two hours (Hasan and Al-Sorkhy, 2014). Extracts were centrifuged at 1000 rpm for two min, the supernatant were pooled. The dried extracts of the herb was prepared in DMEM –LG with 1% L-Glutamine and 1% penicillin streptomycin for further analysis (Archana and Namasivayam, 1999).

Omental adipose tissue from dogs were collected from the undergoing surgery with due consent from Madras Veterinary College Clinics in the Phosphate Buffered Saline (PBS) containing cocktail of antibiotics (Penicillin and streptomycin) with necessary precaution. Apparently healthy domesticated dogs vaccinated regularly undergoing clinical surgeries unrelated to this study were used for adipose tissue collection with due consent as per DBT guidelines. Briefly, adipose tissue was thoroughly washed in PBS with antibiotics.The suspended RBC contamination was removed.The dissociated tissue was treated with 0.075 per cent collagenase (Sigma) in MEM (5 ml/g of tissue) overnight and neutralized the enzyme activity using DMEM

MEMBERS OF RESEARCH FORUM

Author for correspondence :
A. Mangala Gowri, Department of Animal Biotechnology, Centre for Stem Cell Research and Regenerative Research, Madras Veterinary College, Vepery, **Chennai (T.N.) India**

Associate Authors' :
S. Priya and D. Baskaran, Department of Food Science and Technology, College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, **Chennai (T.N.) India**

containing high glucose. Centrifugation at 1200 g for ten minutes yielded pellet and washing of the pellet and resuspension and filteration was done to remove debris to obtain pure cell population. The cells were subjected for magnetic activated cell sorting to obtain a pure population of CD34+ve cells.

The proliferation of Dog adipose derived cells were analysed by seeding at a density of 5000 cells/ cm^2. The confluency reached 90 per cent in control group without herbal supplements was taken as standard to compare the cell proliferation during the supplementation of different herbal concentration ranging from 10 µg/ml-200µg/ml. Cells were counted by trypan blue dye exclusion and the range of concentrations that induced the proliferation of cells was determined.

The cell number concentration cultured with different concentrations of herbal extracts from 10 µg/ml to 200 µg/ml showed a more viable cell population and less cell apoptotic changes were observed in the range of 20 µg/ml to 100 µg/ml. Hence, the concentration used for proliferation analysis of the herb was determined to be

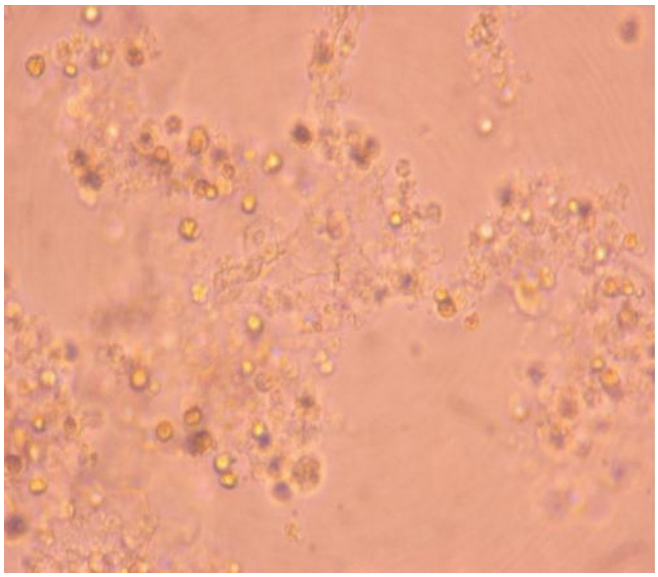

Fig. 1: Cultured dog adipose tissue derived CD34+ve cells (20x)

from 20 µg/ml to 100 µg/ml (Fig 1).

Omental adipose tissue from dogs were collected with due consent from Madras Veterinary College Clinics in the Phosphate Buffered Saline (PBS) containing cocktail of antibiotics (Penicillin and streptomycin) with necessary precaution. Adipose tissue was thoroughly washed in PBS with antibiotics to remove the suspended RBC contamination. collagenase (Sigma) treatment (0.075%) in MEM (5 ml/g of tissue) overnight was done to dissociate the tissue. The dissociated cell pellet was obtained at centrifugation (1200g) for ten minutes and resuspended and filtered was done to remove debris.

The pure cell population was counted and resuspended cells (3000 cells/cm^3) in DMEM - HG with 10% FBS in 25 cm^2 culture flasks. After 24 hrs observed for bifringence and unattached cells were removed. The cells were maintained in growth medium renewed every 3 days until reached 70-80 per cent confluency in 7 days. Representative cell population was stained with DAPI for assessment.

The cells showing 80 per cent confluency were fixed with 2 per cent paraformaldehyde and stained with Mayer's hematoxylin as per standard method and mounted using DPX (di-*n*-butylphthalate in xylene). Oil red "O" staining was done by fixing the cells in formalin (10%) and washed in dd water before incubation in 60 per cent isopropanol for 10 minutes and followed by application of staining and hematoxylin counter staining.

The adipose tissue derived cultured cells were found to be glistening and spherical during initial culture days and assumed spindle morphology during 7^{th} day.

Drug concentration determination for proliferation analysis has been standardized by seeding at a density of 5000 cells/cm^2 to assess the confluency (70- 80%) in control group without herbal supplementation. Cells were counted by trypan blue dye exclusion and the range of concentrations that induced the proliferation of cells was determined to be from 1µg/ml - 200 µg/ml. The methonolic extracts of *Withania somnifera* showed proliferative activity on adipose tissue derived cells.

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