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# Treatment with adipose derived mesenchymal stem cells and their conditioned media reverse carrageenan induced paw oedema in db/db mice

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ABSTRACT

Mesenchymal stem cells are known for anti inflammatory and immunomodulatory activities. The aim of our study was to evaluate the effect of human adipose derived mesenchymal stem cells (hADMSCs) and its conditioned media (CM) on carrageenan induced acute inflammation in db/db mice. We injected $5 \times 10^5$ ADMSCs or the CM in the inflamed paw. We assessed the paw volume, serum IL6 levels and histopathology of the paw to reveal the anti inflammatory effect. We observed a single injection of hADMSCs or CM could reverse the inflammation within 24 h as evidenced by reduction in paw volume, IL6 levels and histological examination. Our result equivocally demonstrates the role of CM in normalising the inflammation better than hADMSCs. This study will pave way for an alternative to anti inflammatory drugs.



## 1. Introduction

Inflammation is a composite biological host reaction to stimuli and is characterized by heat, pain, redness and swelling [1]. A chronic or acute inflammation is strongly associated with diseases such as obesity, diabetes, vascular disease, obstructive pulmonary disease, cancer and so on [2–6].The field of mesenchymal stem cells (MSCs) treatment has prospered recently. There are several reports stating that MSCs are anti inflammatory. Apparently, MSCs play a role as a guardian of inflammation [7]. Paw swelling, or footpad oedema, is a convenient method for assessing inflammatory responses to antigenic challenges and irritants. The protocol described uses carrageenan as the irritant to induce paw oedema. Typically, test compounds are assessed for acute anti-inflammatory activity by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling [8]. The role of MSCs in carragennan induced paw oedema has been established. The role of conditioned media from hADMSCs has not been reported so far. There are some constrains in the usage of stem cells as a treatment of choice regarding source, age of donor, autologous verses allogeneic [9–11]. Using conditioned media would be more convenient and less risky as it is a mixture of all growth factors and cytokines secreted by MSCs. Conditioned media offer a cell free treatment with all the advantages of stem cells without cells. Conditioned media can be stored for a longer period and can be frozen and dried as well; their storage and transport will be easier than MSCs. Hence, usage of conditioned media is user friendly, easy and convenient than using MSCs [12,13]. Here, we investigated the role of hADMSCs and its CM in the recovery of carrageenan induced paw oedema in db/db mice.

## 2. Materials and methods

### 2.1. Study design

db/db mice was a generous gift from Connexios Life Sciences; Bangalore, India. Mice were housed in a controlled environment with research diet-and access to water *ad libitum*. To test the anti inflammatory effect of hADMSCs and its conditioned media; mice model of acute inflammation was developed by inducing paw

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oedema using carrageenan. Animal experiment protocols and experimental procedures were approved by the Connexios Institutional Animal Ethics Committee which are in agreement with the ARRIVE guidelines [14,15].

### 2.2. Acute inflammation

Carrageenan (2%) was prepared in PBS and injected subcutaneously to right paw of the mice for the development of inflammation. Mice were divided in four groups (n = 3) for the treatment viz. PBS control, Carrageenan control, hADMSCs and CM. 50 microlitres of carrageenan was injected to each animal 30 min after the injection of ADMSCs and CM.

### 2.3. Human ADMSCs characterization

Human ADMSCs were procured from ANSA, Bangalore, India. ADMSCs were grown in knock out DMEM media with 10% fetal bovine serum. The cells were trypsinized, fixed, stained and characterized by flow cytometry using the mesenchymal markers. CD105 and CD90 were used as positive markers for MSCs; CD34 and HLA-DR were used as a negative marker. Cells were also differentiated to adipocytes, Osteocytes and chondrocytes to confirm the trilineage potential of MSCs.

### 2.4. hADMSCs and CM preparation

hADMSCs were grown as mentioned above. Once the cells reach 100% confluency, the conditioned media was collected from the same passage (Passage 3) centrifuged at 1800 rpm for 10 min, filtered, diluted 50% with the growth media and used for treatment. 50% CM was used for the treatment following Hao et al. They showed that CM derived from adipose tissue exhibits reduced glutamate-induced neuronal injury in a concentration-dependent manner, the maximum protective effect being at 50% CM. They further state that lower than 50% CM or at 70% CM, protective effects were less evident. 100% CM do not mediate any protection. [16]. Cells were trypsinized, counted and $5 \times 10^5$ cells were used as a suspension for subcutaneous injection.

### 2.5. Plethysmometer measurement

Changes in the paw volume were measured using a digital plethysmometer (INCO, Niviqure). Paw volume was measured at 24 h from the time of treatment.

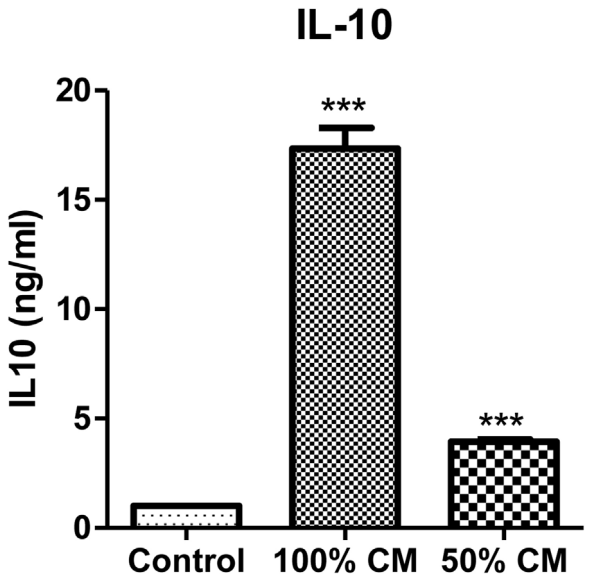


**Fig. 2.** Graphs representing levels of IL10 in the conditioned media.

### 2.6. IL6 measurement

One of the most prominent proinflammatory cytokines to rise in the blood is IL6 when affected with inflammation. Serum IL6 was measured at the end of 24 h of treatment using Quantikine ELISA kit from R&D systems Inc. Minneapolis, USA as per the manufacturer's instructions.

### 2.7. IL10 cytokine assay

IL-10 an anti-inflammatory cytokine was measured in the conditioned media (both in diluted and undiluted CM) using Human IL-10 ELISA kit from BD biosciences, San Diego, USA.

### 2.8. Histological examinations

Paw tissues were fixed in formalin and 4 μm sections were stained with hematoxylin and eosin (H&E) stain. The images were captured using inverted microscope (Nikon Eclipse TE2000-5, Japan).

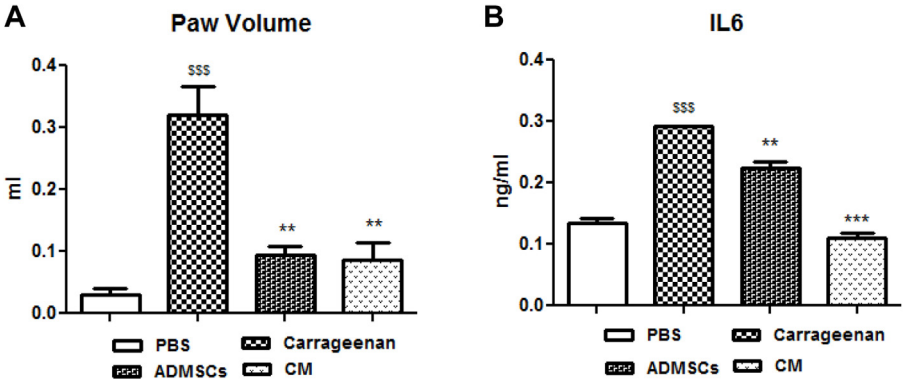


**Fig. 1.** '$' is compared to the PBS control and '*' is compare to the Carrageenan treatment.
(A) Measurement of paw volume in ml using plythesmometer. (B) Serum IL6 ELISA measured as ng/ml.