The Effect of Camel Placenta Extract on The Degraded Osteoarthritic Joints: *In vivo* Evaluation

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INTRODUCTION

Osteoarthritis (OA) is the most common chronic condition of the joints, affecting 53.3% of male and 60.9% of female in Saudi Arabia, resulting in a reduction of the quality of life. Traditional therapeutic approaches for the treatment of OA focus on symptoms alleviation, while the new approaches are directed toward regenerative medicine, including fibroblast growth factor-18, mesenchymal stem cells, platelets-rich plasma, and hyaluronan. However, these treatments are very costly.

This study aims to evaluate the effect of camel placental extract (CPE) on the damaged osteoarthritic cartilage, with a less economic burden.

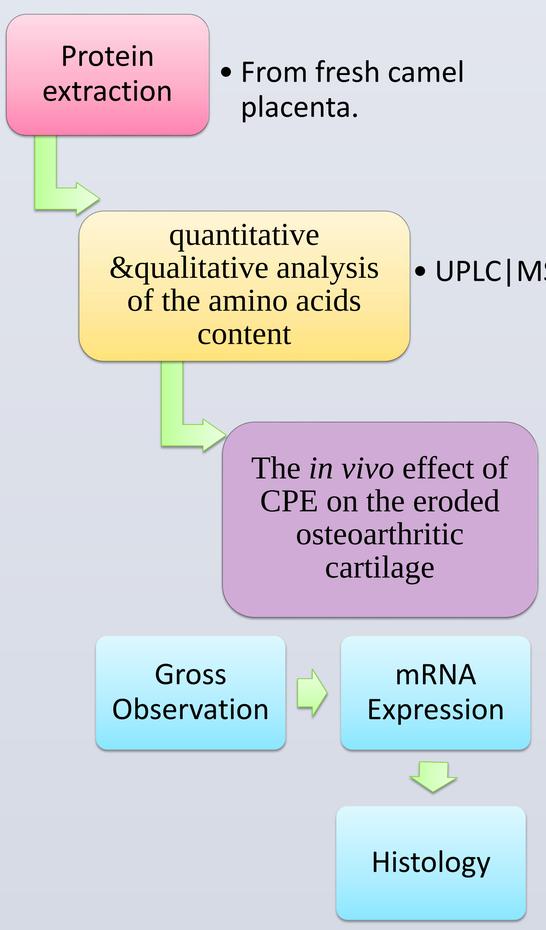
OBJECTIVES

1.To identify the amino acid sequence of the camel placenta extract.

2.To evaluate the protective effect of the CPE on osteoarthritic cartilage *in vivo*.

3.To publish the results in ISI journals and apply for a patent. As well, The final results will be publicly announced to the stakeholders.

MATERIALS & METHODS



Protein Extraction:

Fresh camel placenta was washed then cut into small pieces. Each piece was buffer (PH=7.4) and homogenized using a tissue homogenizer before centrifugation. After centrifugation, the separated lipid layer was removed, and a clear supernatant was obtained. The concentration of the supernatant was 5.7 g/mL according to Bradford assay. This supernatant will be referred to as “the total protein extract”.

Amino Acid Quantitative Analysis:

A portion of the total protein extract was hydrolyzed in order to quantify the amino acids content that exist in the CPE.

The hydrolysate was injected into UPLC-MS/MS separation system instrument for both qualitative and quantitative analysis of the amino acids present in the extract.

***In Vivo* Study:**

OA was induced in rats by intraarticular injection of Monoiodoacetate then rats were randomly divided into four groups to start treatment. Each group received a daily intraarticular injection of one of the following treatment once a day for 14 days.

Table 1. Different treatment received by OA bearing rats:

Group Number	Drug
Group I	**Diclofenac 3mg/kg**
Group II	**CPE 0.6 mL/kg**
Group III	**CPE 0.8 mL/kg**
Group IV	**Control (no treatment)**

RESULTS

1. UPLC/MS

The analysis results revealed that the CPE consists of sixteen amino acids with isoleucine and valine composing the majority (Figure.1).

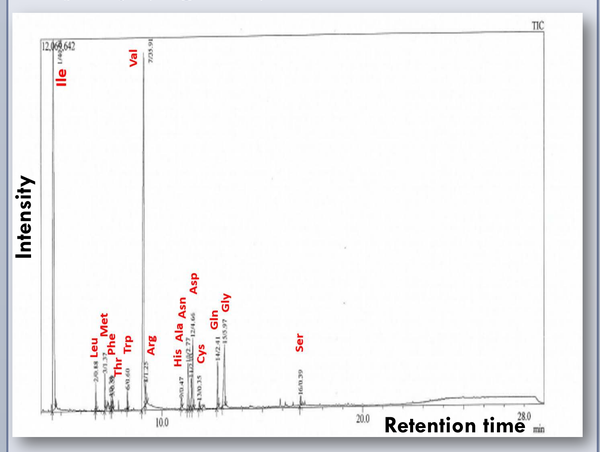


Figure 1. UPLC\MS chromatogram represents the quantitative analysis of amino acids were found in CPE.

2. Gross Observation

2.1 Von Frey test: is an indicator of mechanical sensitivity (allodynia). Rats treated with CPE showed more sensitivity to Von Frey monofilament hairs. Thus, a more effective treatment compared to declofinac. (Figure 2).

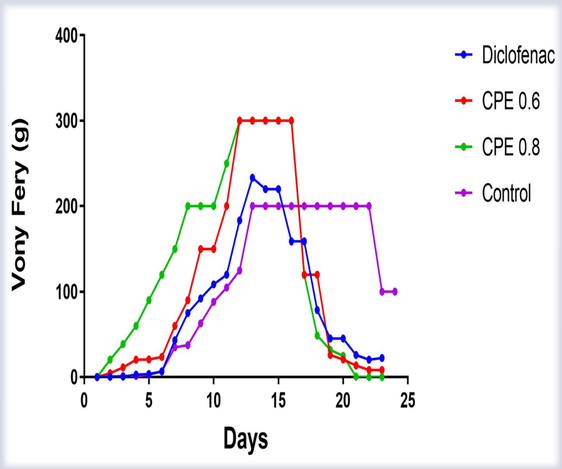


Figure 2. Von Frey test results.

2.2 Weight Borne. The weight borne of the affected hind limb was reduced post MIA injection for all groups indicating induction of OA. After treatment, the weight borne was increased in 0.6 & 0.8 CPE treated groups comparing to diclofenac treated group (Figure.3), which means CPE was capable of attenuating weight –bearing deficits in rats.

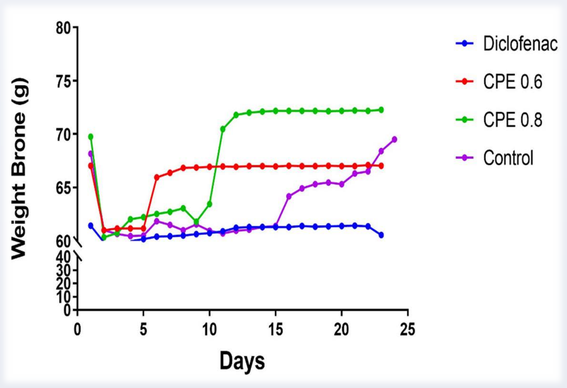


Figure 3. Weight borne changes post MIA injection and after treatment.

2.3 Relative Weight of Rats.

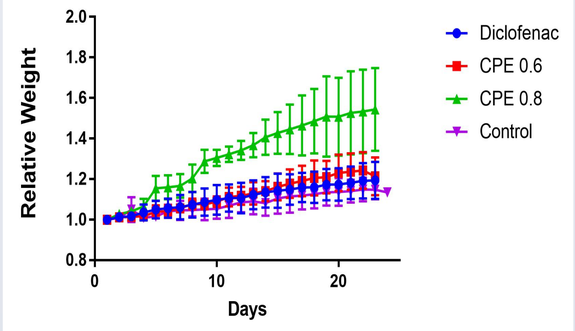


Figure 4. Relative weight measurements chart.

2.4 Skin Irritation Comparison.

Diclofenac treated rats (Figure 5, left) showed irritation and swelling at site of injection while CPE treated rats (Figure 5, right) showed neither irritation nor swelling.

Figure 5. Skin irritation comparison.

3. mRNA Expression of MMPs

Matrix metalloproteinases (MMPs) are a group of enzymes over expressed in OA, causing progressive degradation of the joints. Among the MMPs, gelatinase A (MMP-2) and gelatinase B (MMP-9) has an influence over the onset and progression of osteoarthritis affecting the subchondral bone remodeling process. The real time RT PCR study of the MMP-2 and -9 gene expression proofed that CPE caused a reduction in the expression levels, which was obvious at day 5, but was more pronounced at day 14 (Figure 6). MMP-2 was reduced by the different doses of CPE and by diclofenac to a comparable level (Figure 6, A). While 0.6 CPE and diclofenac reduced MMP-9 levels slightly more than 0.8 CPE (Figure 6, B).

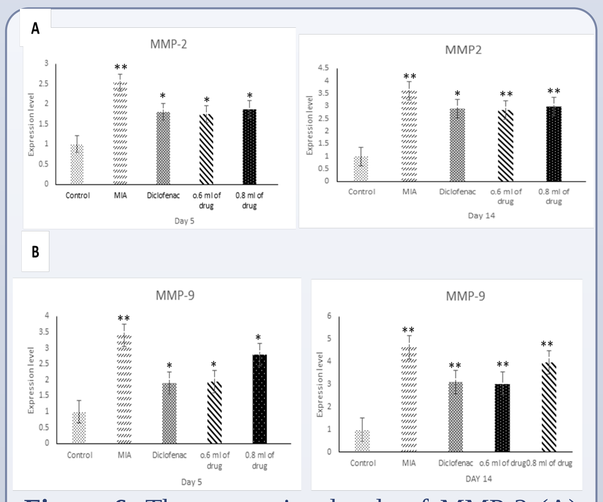


Figure 6. The expression levels of MMP-2 (A) and MMP-9 (B) in rat plasma samples obtained at day 5 and day 14 of treatment post MIA injection, the control group is the group free from the disease.

3. Histology

The 0.6 ml CPE initiated joint healing represented in the form of thin membrane covering the injured area of the cartilage. 0.8 ml CPE showed almost complete healing of the injured area that appears to mimic the normal control rat. Diclofenac treated group showed cartilaginous injury and inflammatory cellular infiltration (non-complete healing). (Figure 7).

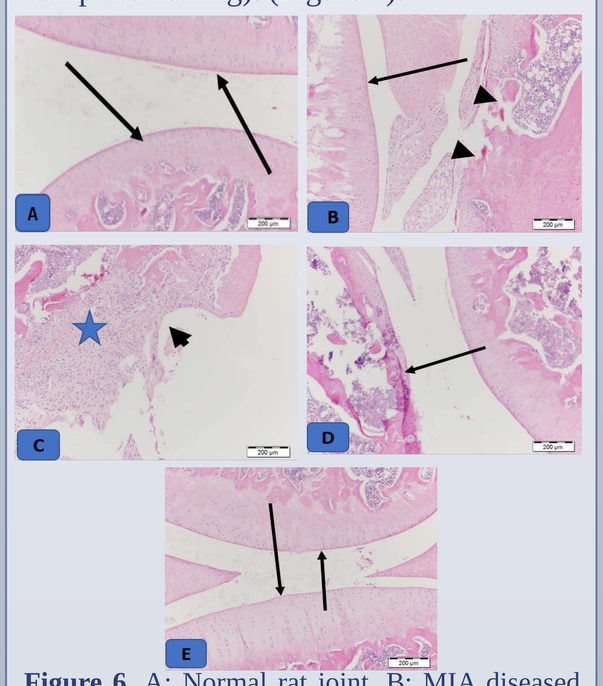


Figure 6. A: Normal rat joint. B: MIA diseased joint without treatment. C: Diclofenac treated joint. D: 0.6 CPE treated joint. E: 0.8 CPE treated joint.

CONCLUSION

CPE intraarticular injection in MIA-induced OA in rats proofed its ability to regenerate the eroded osteoarthritic joints without causing irritation nor swelling at the site of injection. CPE might owe its efficacy to its ability to make histopathological changes on the eroded cartilage and reduce the gene expression levels of MMP-2 & -9. CPE intraarticular injections might be a promising new economical approach for the management of OA.

REFRENCES

Kim, J, Kim T, Park S, Kim H, Kim S, Lee S, Lee S. Protective Effects of Human Placenta Extract on Cartilage Degradation in Experimental Osteoarthritis. Biological & Pharmaceutical Bulletin. 33(6):1004-1010(2010).

ACKNOWLEDGEMENTS

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