

# EXPRESSION MODULATION OF TUMOUR NECROSIS FACTOR ALPHA AND TUMOUR NECROSIS FACTOR RECEPTOR - 1 -7GENES IN BREAST CANCER CELL LINES (MCF-7) BY SOME SELECTED INDIGENOUS CYTOTOXIC PLANTS



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## SUMMARY

Plants have increasingly played a significant role in the treatment of cancer and infectious diseases for several decades. Thirty percent of all cancers in women occur in the breast making it the most commonly diagnosed female cancer. Cytotoxic properties of 80% aqueous-ethanol extract, n-hexane, chloroform, ethylacetate, detannified, and tannin fractions of selected study plants were evaluated for Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and tumor necrosis factor receptor (TNFR1) gene modulations. Study plants includes-*Curculigo pilosa's* Rhizomes, *Icacina trichantha's* Leaves, *Anthocleista djalensis's* Leaves, *Gladiolus psittacinus's* bulbs, *Tapinanthus bangwensis's* leaves, and *Spilanthes ficalis's* leaves. Following brineshrimp lethality assay, fractions of each plant (detannified *Icacina trichantha*, crude extract of *Curculigo pilosa*, hexane fraction of *Spilanthes ficalis*, detannified *Anthocleista djalensis*, hexane fraction of *Tapinanthus bangwensis* and crude extract of *Gladiolus psittacinus*) with the lowest LC<sub>50</sub> were selected for the gene expression study. Concentrations that are five-fold lower than the LC<sub>50</sub> of the six fractions were inoculated in triplicates into MCF-7 cells for 48hours, after which the expression of TNF- $\alpha$  and TNFR1 genes were examined. . TNFR1 gene expression was not observed in MCF-7 cell lines while TNF- $\alpha$  expression was induced significantly ( $P < 0.05$ ) by the test fractions. In conclusion, the test fractions in this study do not induce apoptosis via the molecular mechanism of TNF- $\alpha$  and TNFR1 expression but may support immunological activation due to the significant high levels of TNF- $\alpha$  gene expression.

## INTRODUCTION

The use of herbal intervention in the treatment of tumors is widespread in all regions of the developing world even though there are insufficient data on their possible molecular mechanisms of action. The use of gene expression profiling has proven to be an important tool in the understanding of cancer biology and prediction of prognostic factors (Arango *et al.*, 2013). Evaluating gene expression modulations due to anti-tumour agents from medicinal plants may provide useful information in understanding the underlying molecular mechanism of action of these phytochemicals; hence, validating their anti-tumour efficacy (Kang *et al.*, 2005). In an effort to studying part of the molecular mechanisms of the anti-neoplastic effect of such plants; *Curculigo pilosa* Cpi, *Gladiolus psittacinus* Gps, *Anthocleista djalensis* Adj, *Tapinanthus bangwensis* Tba, *Icacina trichantha* Itr, *Spilanthes ficalis* Sfi, were investigated for their modulatory effects on TNF- $\alpha$  and TNFR1 genes.

## METHODS

### Preparation of Plants' Crude Extracts and Fractions

Each of the plant extract was prepared by maceration in 80% aqueous-ethanol and the plant extracts were individually fractionated by organic solvent extraction (n-hexane, chloroform & ethylacetate) partitioning with water as previously described by Samuel *et al.*, (2013)

### Cytotoxicity Assay

Brine shrimp cytotoxicity assay was used to screen the thirty six (36) fractions (crude extract, n-hexane, chloroform, ethylacetate, detannified, and tannin fractions for each of the six plants)

**RNA Extraction and cDNA Synthesis;** using Qiagen RNeasy kit (USA) and Jena Bioscience SCRIPT® Reverse transcriptase kit (Germany) respectively

**TNF- $\alpha$  and TNFR Gene Quantification.** Base pairs used for TNFR1 fwd 5'-GTG CCT ACC CCA GAT TGA GA-3', and TNFR1 revs 5'-CTT CAA GCT CCC CCT CTT TT-3', B- actin revs 5'- ACA TGA TCT GGG TCA TCT TCT C-3', and B-actin fwd 5'-GGC ATG GGT CAG AAG GAT TC-3'

. Beta actin gene was used as the internal control (endogenous gene). PCR amplicons were run on 1.8 % agarose gel electrophoresis in Tris-Acetate EDTA buffer at 120 V for 25 minutes. Gel Images were captured and analyzed using Gelanalyzer (USA).

**Data Analysis,** Relative densitometric intensity of the PCR amplicon bands was done using Gelanalyzer software. The intensity values were computed into Graphpad Prism 5® software to calculate the significant differences between the Control and test groups at 95% confidence interval.

## RESULTS

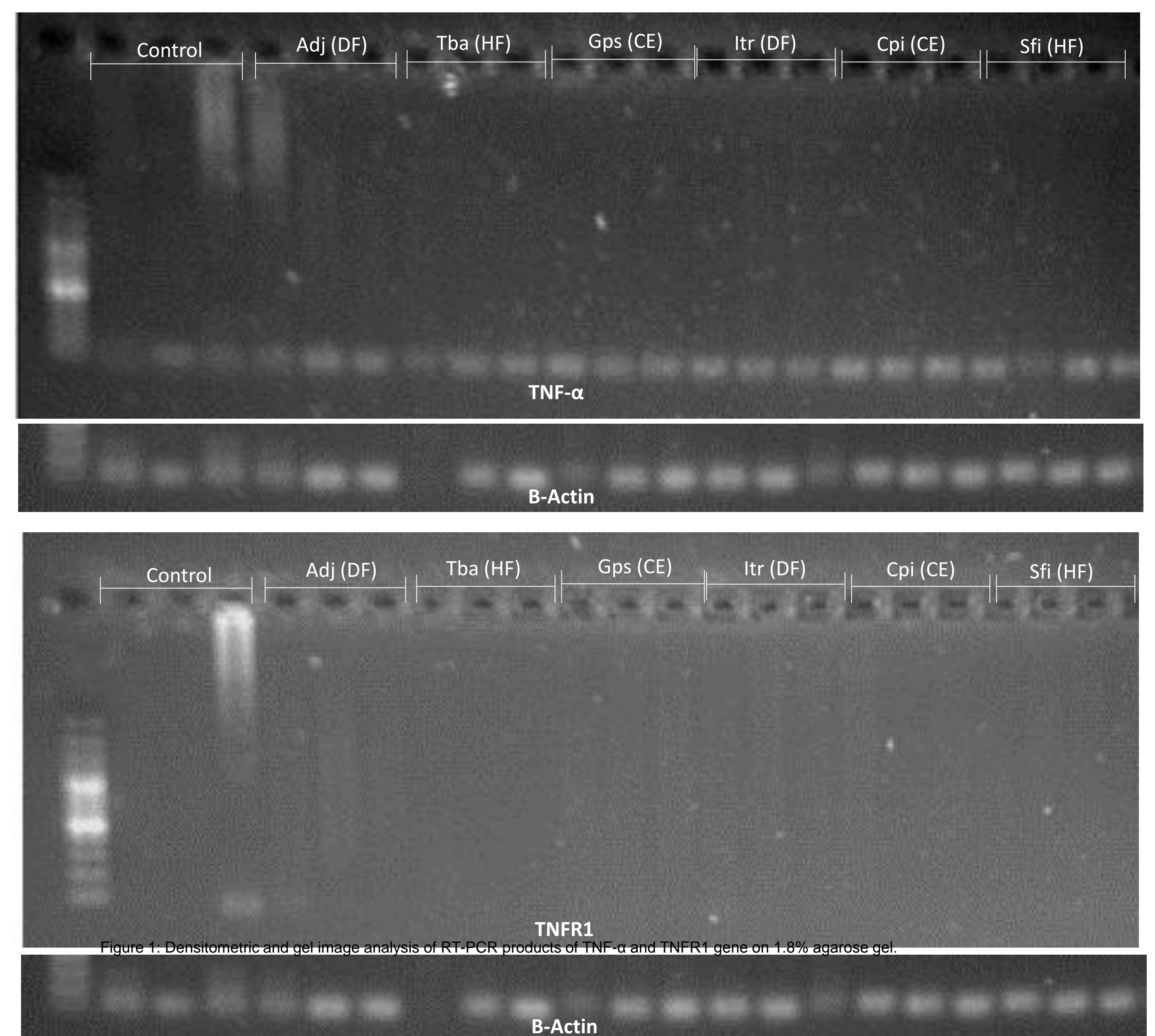
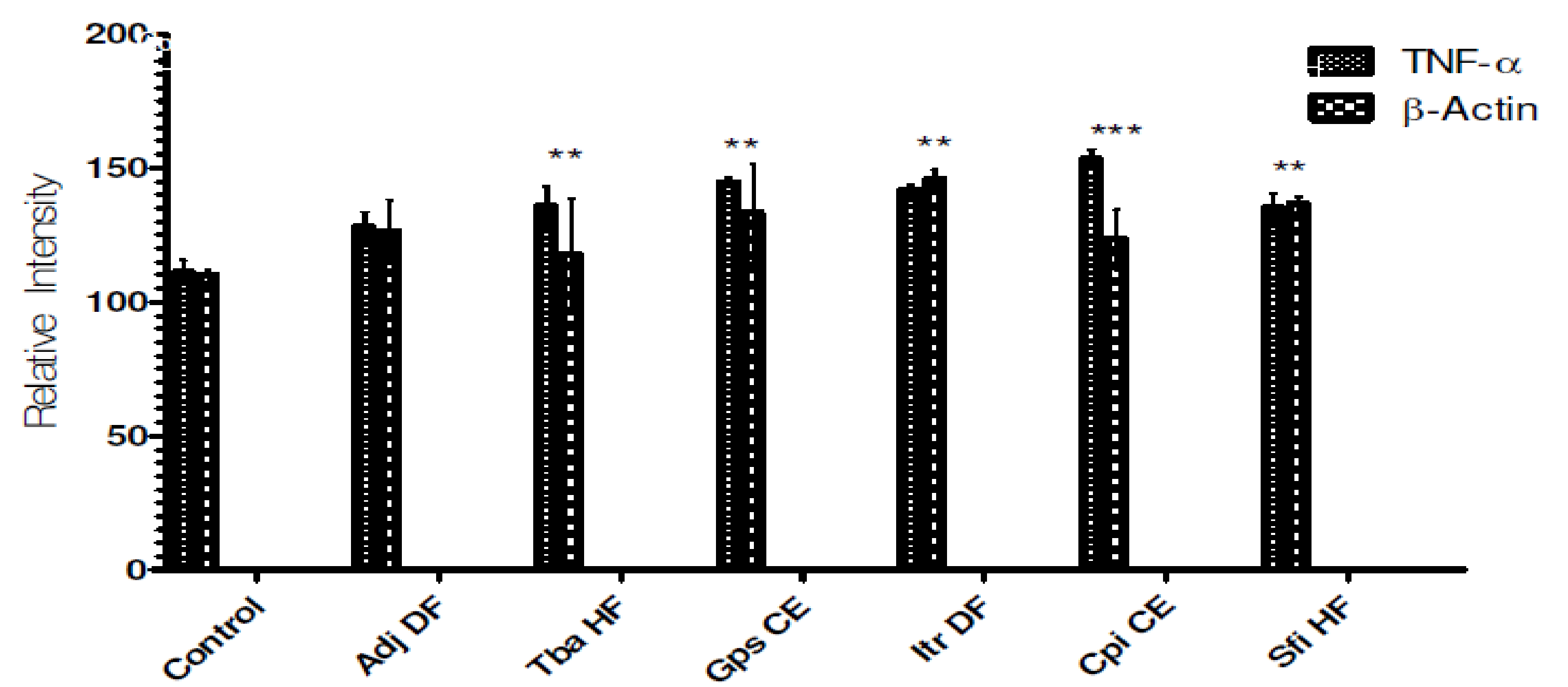


Figure 1: Densitometric and gel image analysis of RT-PCR amplicons for (A.) TNF- $\alpha$  gene on 1.8% agarose gel (B.) error bar charts showing relative intensities of each band; testing was done in triplicates an values are Mean  $\pm$  SE.  $P < 0.05$  is considered significant versus control group (C.)  $\beta$ -Actin amplicons on 1.8 % agarose gel.

## CONCLUSION

This result suggests that the test fractions in this study do not induce apoptosis via the molecular mechanism of TNF- $\alpha$  and TNFR1 expression but may support immunological action due to the significant high levels of TNF- $\alpha$  gene expression

## REFERENCES

- Arango, B. A., C. L. Rivera, S. Gluck, 2013. . *Am J. Transl Res.* 5(2): 132-138
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