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Investigation of in-vitro anti-inflammatory Activity of Methanolic Extract of Carica Papaya

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Abstract—*Methanolic extract of leaves of Carica papaya (Family : Caricaceae) was evaluated for its anti-inflammatory activity using bovine serum albumin denaturation assay and human red blood cell membrane stability test. The results were compared with diclofenac sodium as the standard. Linear regression analysis was used to determine IC50 values. At concentration of 100 µg/ml diclofenac showed 66% BSA inhibition while the extract showed 66.67% inhibition. The extract showed 88.2 % protection in HRBC membrane stability assay while diclofenac showed 84.27%.*
The results suggest that the methanolic extract of Carica papaya has a promising therapeutic potential.

1. INTRODUCTION

Inflammation is a defensive process, which indicates body's efforts to guard itself against and eliminate harmful stimuli, allergens, pathogens that could cause harm to the body. It is a part of the natural healing process of the body and is generally characterized by pain and encompasses molecular events like vascular permeability increases along with, increase of protein denaturation and destabilization of membranes.

A number of natural sources are being explored to help ease the symptoms of inflammation to produce an effect comparable to that of non-steroidal anti-inflammatory drug (NSAID) while reducing the number of side-effects as caused by NSAIDs. In spite of the relief that these allopathic treatments provide, they also bring along opposing effects irritable bowel syndrome, re-distribution of fat in the body, asthma, inhibition of platelet function and anaphylactic reactions in patients.

Carica papaya commonly known as papaya is a tropical fruit and while it is considered tasty it also harbors immense therapeutic potential. Parts of the plants right from its root, stem, peel, seeds, bark and the fleshy fruit itself are being studied to address many medical conditions. This study focuses on the leaves of this plant with the aim of proving it as a potential drug to alleviate symptoms of inflammation.

2. MATERIALS AND METHODS

2.1 Materials

Methanol, fresh leaves of *Carica papaya*, Diclofenac Sodium, Bovine Serum Albumin, Phosphate buffer (50mM, pH 6.6), isosaline, hyposaline and Alsevier's solution.

2.2 Methods

2.2.1 Inhibition of BSA denaturation Assay. Different concentrations of freshly prepared methanol extract of the leaves of C.papaya i.e 5, 10, 25, 50, 100 µg/ml were prepared using isosaline. Each of these test samples were incubated with 1.8ml of 1% BSA solution at 37°C for 20 minutes. This was followed by a second incubation at 57°C for 15 minutes. Diclofenac sodium was used a positive control. The negative control was taken without the drug and extract. The samples were cooled and absorbance was checked at 660 nm.

The % of denaturation was calculated using –

(O.D of control – O.D of Test)/ O.D of control x 100

2.2.2 HRBC Membrane stability Assay. Fresh blood samples were collected from healthy individuals, immediately mixed with Alsevier's solution and centrifuged at 3000 rpm for 15 minutes. The packed cells were washed using isosaline. The packed cells and isosaline were mixed in calculated amounts to prepare a 10% solution of the cells in isosaline. Again, different concentrations of methanol extract of the leaves of C.papaya in isosaline were incubated along with 0.5 mL of the extract, 1 mL phosphate buffer, 2 mL hyposaline and 0.5 mL HRBC suspension at 37°C for 30 minutes. After incubation, they were centrifuged at 3000 rpm for 20 minutes. Absorbance of the samples was measured at 560 nm. Diclofenac sodium was used as the positive control and control was taken without the extract served as negative control.

The % protection was calculated using-

(O.D of control – O.D of Test)/ O.D of control x 100

3. RESULTS

3.1 BSA denaturation inhibition assay

The inhibition of BSA denaturation by the methanolic extract is shown in Fig 1. The percentage BSA denaturation in the case of extract and standard, Diclofenac, at 100µg/mL concentration, was 66.67% and 66% respectively

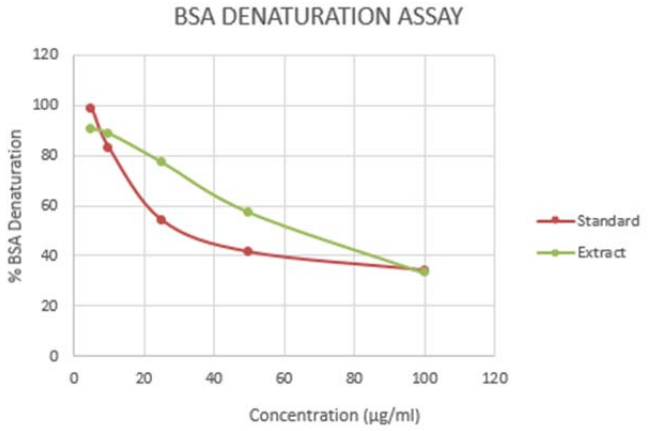
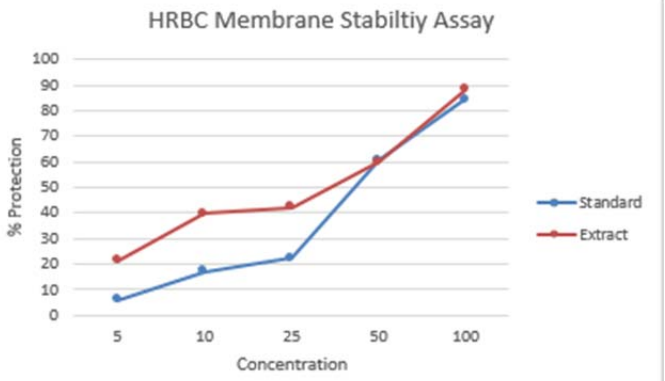


Fig. 1: BSA Denaturation Assay

3.2 HRBC membrane stabilization assay

The membrane stabilizing properties of methanolic extract and diclofenac are shown in Fig 2. The percentage protection in the case of extract and standard, Diclofenac, at 100µg/mL concentration, was 88.2% and 84.27% respectively.



4. DISCUSSION

In this investigation, the anti-inflammatory activity of the methanolic extract of the leaves of C.papaya evaluated using *in vitro* assays induced by chemical mediators.

The denaturation of proteins is a proven cause of inflammation and hence, this assay was implemented to evaluate the properties of the extract in protecting the protein from the denaturation process. At 100µg/mL both the leaf extract as well as standard show almost similar results with % protection of 66.67% and 66% respectively.

The inhibition of hypo-tonicity induced HRBC membrane lysis was taken as a marker to gauge the effectiveness of the methanolic extract and further evaluation at different concentrations showed that at 100µg/mL concentration the leaf extract shows activity at par with the positive control i.e. dicofenac. The % protection of leaf extract was 88.2% and that of diclofenac was 84.27%.

5. CONCLUSION

The methanolic extract of *Carica papaya* leaf, exhibited substantial anti-inflammatory activity when compared with the standard NSAID. The information on the IC50 values of the extract in the assays studied i.e., BSA denaturation inhibition (69.5 µg/mL of extract vs. 57.94 µg/mL of standard) and HRBC membrane stabilization (3.489 µg/mL of extract vs. 3.597 µg/mL of standard) shows its potential as an anti-inflammatory drug.

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