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Research Article

**IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF GLYCINE
MAX SEEDS****Bhupendra Kumar*¹, Meenakshi Ghildiyal ², Yogesh Tiwari ³, Deepika Chauhan ⁴,
Amandeep Singh ⁵**¹Assistant Professor, Dev Bhoomi Institute of Pharmacy & Research, Dehradun²Assistant Professor, Dev Bhoomi Institute of Pharmacy & Research, Dehradun³Assistant Professor, Dev Bhoomi Institute of Pharmacy & Research, Dehradun⁴Research Scholar, Dev Bhoomi Institute of Pharmacy & Research, Dehradun⁵Associate Professor, Dev Bhoomi Institute of Pharmacy & Research, Dehradun**Abstract:**

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective immune vascular response that involves immune cells, blood vessels, and molecular mediators. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the inflammatory process, and to initiate tissue repair. Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituent of activated neutrophils such as bacteria enzyme and proteases which cause further tissue inflammation and damage.

Keywords: HRBC, Anti-inflammatory**Corresponding Author:****Mr. Bhupendra Kumar**

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INTRODUCTION:

Inflammation is a protective response for the purpose of removal of exogenous and endogenous harmful substances produced by injurious stimuli and is a part of the healing process in wounded tissues. Since pro inflammatory cytokines such as tumor necrosis factor alpha (TNF- α), interleukin(IL)-1 and IL-6, lipid mediators, proteases, and oxidants produced during the typical response, can cause damage to normal tissues regardless of how and where the inflammatory response is triggered, the substances involved in the inflammatory response need to be tightly regulated. If the scavenging reaction is delayed, the inflammatory response may evolve into a variety of chronic inflammatory diseases, such as atherosclerosis, rheumatoid arthritis, asthma, and neurodegenerative diseases [1]. The four characters, sign and symptom of inflammation are redness, pain, heat, and swelling [2]. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. The chronic inflammation is long term inflammation. Its signs and symptoms are seen for a several months and even years. It is associated

with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis) and fibrosis [3].

Plant Profile [4]

Biological name: *Glycine max*

Family: Fabaceae

Chemical constituents:

It is rich source of carbohydrates, fats, vitamins, and minerals. Soya has high content of quality proteins. Along with calcium it also contains iron, magnesium and potassium [5].

- It contains no cholesterol and helps to lower blood cholesterol level [6]
- It also contains Iso-flavones i.e. genistein and daidzein [7]



Fig.1: Leaves & Seeds of *Glycine max* [8]

MATERIAL AND METHODS: [9]**Collection of Plant Material**

The *Glycine max* seeds were purchased from local market of Dehradun. After drying for 2 weeks seeds of *Glycine max* seeds were crushed to powder by Mortar pestle.

Preparation of Extract**Aqueous, Methanolic & Hydro alcoholic extract**

100gm of the powdered drug was soaked with a little of distilled water for the aqueous extract & a little of methanol for methanolic extract & ethanol & water in a ratio of 50:50 for hydro alcoholic extract respectively in a stainless steel closed container. After an hour 200 ml of water was added to the moistened drug and it was allowed to macerate for 7 days with occasional shaking. After a week the liquid was filtered with the help of a muslin cloth and the drug material was pressed to liberated more menstrum form the marc. Both the extracts were mixed and the liquid evaporated to get a brown colored aqueous extract. The percentage yield was calculated for the extract which was later used for the study.

Evaluation of anti-inflammatory activity**Human Red Blood Cell (HRBC) membrane stabilization method**

The principle concerned in this method is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. Blood was collected (2ml) from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl in distilled water) and centrifuged at 3000 rpm. The packed cells were washed with isosaline solution and a 10% suspension was prepared with normal saline and kept at 4°C. Different concentration of *Glycine max* extracts (100µg/ml, 200µg/ml) and control distilled water instead of hypo saline to produce 100% hemolysis) were separately mixed with 1 ml of phosphate buffer, 2ml of hyposaline and 0.5 ml of 10% HRBC suspension were added to prepare. All the assay mixture were incubated at 37°C for 30 min. and centrifuged at 3000 rpm for 20 min. at 25°C, and hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula [10].

Absorbance of control - Absorbance of test

$$\% \text{ stabilization} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

RESULTS:

S. No.	Types of extract	Concentration(µg/ml)	Absorbance	% Inhibition of Denaturation
1	Control	-----	0.4950±0.055	-----
2	Water soluble	100 (µg/ml)	0.1475±0.079	70.2
3	Water soluble	200 (µg/ml)	0.1385±0.087	72.02
4	Alcoholic	100 (µg/ml)	0.1395±0.073	71.81
5	Alcoholic	200 (µg/ml)	0.1235±0.076	75.05
6	Hydro alcoholic	100 (µg/ml)	0.1690±0.111	65.85
7	Hydro alcoholic	200 (µg/ml)	0.1325±0.087	73.23
8	Diclofenac	100 (µg/ml)	0.2260±0.142	54.34
9	Diclofenac	200 (µg/ml)	0.1555±0.112	68.58

DISCUSSION:

The HRBC membrane stabilization method was used for the In-vitro anti-inflammatory activity of the different extracts of seeds of *Glycine max*. All the extracts showed more activity than the standard drug. Alcoholic extract showed maximum activity (i.e. 75.05% inhibition of denaturation in hypotonic solution) at the concentration of 200µg/ml as the comparison of the standard Diclofenac 200 (µg/ml) showed 68.58 % inhibition of denaturation.

CONCLUSION:

Alcoholic extract of *Glycine max* seeds exhibited maximum membrane stabilization effect by inhibiting hypo tonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogues to the lysosomal membrane and its stabilization implied that the extract may be well stabilizing lysosomal membrane. From the above study it was concluded that the Alcoholic extract of *Glycine max* has significant membrane stabilization property. Hence proper isolation of the active constituent might help in the finding of new lead compound in the fields of anti-inflammatory drug. Research studies related to active constituent enzyme expression (cox-2, lipoxigenase) are necessary to understand the mechanism of action in relation to the observed anti-inflammatory activity.

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