IN VITRO ANTI-INFLAMMATORY ACTIVITY OF SEED EXTRACT OF Zea Mays (L.)

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Abstract
The seed of Zea mays (Linn) (Family : Poeceae) commonly known as sweet corn. It is widely used as an ethanomedicine in India. It is prescribed for a wide variety of ailment like diuretic and mild stimulant. Preliminary phytochemical studies revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, and glycosides. The anti-inflammatory activity of Zea mays ethanolic extract was done by inhibition of protein denaturation and Human Red Blood Cell membrane stabilization (HRBC) Invitro methods. The ethanolic extract of Zea mays was subjected to invitro inhibition of protein denaturation in various concentration i.e. 100, 200, 400, 800, 1000 µg/ml. HRBC method was also used for the estimation of anti-inflammatory activity from in various concentrations 100, 200, 400, 800, 1600 µg / ml. Zea mays etanolic extract exhibited a concentration dependent inhibition of protein (Albumin) denaturation. The stabilization of HRBC membrane showed a concentration dependent anti-inflammatory activity. It is concluded that these studies of Zea mays seed is an good anti inflammatory agent.
Key words: Anti-inflammatory, Phytochemicals, Human red blood cell membrane stabilization, inhibition of protein denaturation, Zea mays(Linn).

INTRODUCTION
Inflammation is the means by which the body deals with insult and injury. Result may be caused: mechanically (e.g., by pressure or foreign bodies) chemically (e.g., by toxins, acidity, alkalinity) physically (e.g., by temperature, by internal processes (e.g., uremia) and by microorganism (e.g., bacteria, virus, parasites. Inflammation is a complicated and not fully understood communication between cellular and humoral elements[1]. Inflammation rids the body of the foreign matter and disposes of damaged cells, and initiates wound healing inflammation is controlled by mast cells that are in close proximity to autonomic nerves. Mast cells are a constituents of connective tissues containing large granules that contain heparin, serotonin, bradykinin, and histamine. These substances are released from the mast cell in response to injury and infection, and by their degranulation, they control most of the processes of inflammation[2]. Rheumatoid arthritis is one of the challenging disorders associated with inflammatory condition. Various molecule have been isolated from the plants which have been proven very effective in such condition. Drugs which are in use presently for the management if pain and inflammatory conditions are either narcotics. Eg. Opiods or Nonnarcotics, salicylates and corticosteroids, hydrocortisone.
All of these drugs present well known side and toxic effects. It is well documented that these non steroidal anti-inflammatory drugs (NSAIDs) produce intestinal tract ulcers, and erosions of the stomach lining and intestinal tract in 30-50 percent of cases[3]. As a result of these side effect, NSAID use is associated with 10,000 - 20,000 deaths per year in the U.S. Even the new COX-2 inhibitor drugs have only been reported to reduce intestinal tract damage by 50 percent, and their toxicity to the liver and kidneys is still review [4].

MATERIALS AND METHODS
Collection of plant materials
The fresh seed of *Zea mays* Linn were collected from local shop, Mannargudi, Thiruvarur district, Tamilnadu, India. The collected material were cleaned, shade dried and coarsely powdered. The plant material was powdered and used for further studies.

Extraction
The dried seeds are powdered using mixer grinder, 1000 gm of the powdered seeds was packed evenly in the soxhlet extractor and subjected to extraction with ethanol. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a dessicator. The crude extract was used for further invtro anti inflammatory activity.

Phytochemical screening of Qualitative analysis
Ethanolic extract of *Zea mays* seeds are subjected to preliminary screening of Phytochemical constituents. The procedures were analyzed qualitatively by the method of Sofowora, 1993; Harbione,1973 [5,6]

In vitro anti-inflammatory activity
Inhibition of albumin denaturation
The following procedure was followed by Saleem et al. (2011) [7] for evaluating the percentage of inhibition of protein denaturation:

- **Control solution (50 ml)**
  2 ml of egg albumin, 28 ml of phosphate buffer (pH 6.4) and 20 ml distilled water.

- **Standard drug (50 ml)**
  2 ml of egg albumin, 28 ml of phosphate buffer and various concentration of standard drug (Aspirin) concentration of 100, 200, 400, 800, and 1000 µg/ml.

- **Test solution (50 ml)**
  2 ml of egg albumin, 28 ml of phosphate buffer and various concentration of plant extract (*Zea mays* ethanolic seed extract) concentration of 100, 200, 400, 800, and 1000 µg/ml.

All of the above solutions were adjusted to pH using a small amount of 1N HCl. The samples were incubated at 37º C for 15 minutes and heated at 70º C for 5 minutes. After cooling the absorbance of the above solutions percentage inhibition of protein denaturation was calculated using the following formula [8,9].

\[
\text{Percentage inhibition} = \left( \frac{V_t}{V_c} - 1 \right) \times 100
\]

Where,
\[V_t = \text{Absorbance of test sample}\]
\[V_c = \text{Absorbance of control}\]

Human red blood cell membrane stabilization method (HRBC)
The HRBC membrane stabilization has been used as a method to study the anti-inflammatory activity. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of sterilized Alsever's solution (2% dextrose, 0.8%sodium citric acid, and 0.42% sodium chloride in water) the blood was centrifuged at 3000 rpm and packed cells were washed with isosaline(0.85%,pH 7.4) and a 10 % v/v suspension was made with isosaline.

The assay mixtures contain the drug (at various concentration as mentioned in the table). 1ml phosphate buffer (0.15 M, pH 7.4) and 2ml of hyposaline(0.36%)and 0.5 ml HRBC
suspension. Aspirin was used as reference drug. Instead of hyposaline 2 ml of distilled water was used in the control. All the assay mixture were incubated at 37° C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated by a spectrophotometer at 560 nm. The percentage of hemolysis was estimated by assuming the hemolysis produced in the presence of distilled water as 100% [10,11]. The percentage of HRBC Membrane stabilization or protection was calculated by using the following formula:

\[
\text{Percentage protection} = \left[100 - \left(\frac{\text{optical density sample}}{\text{optical density control}}\right)\right] \times 100.
\]

**RESULTS**

The present study carried out the *Zea mays* L. seed sample revealed the presence of medicinally active metabolites. The phytochemical evaluation of the seed extract was done for the presence of alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids, coumarins, glycoside, and reducing sugar and absent of quinone, phenols and phlobatannins (Table. 1).

*Zea mays* hair founds in Coimbatore has been reported to contain flavonoids, alkaloids, phenols, steroids, glycosides, carbohydrate, terpenoids and tannins [12].

**Table 1: Phytochemical Screening of Ethanolic Seed Extract of *Zea Mays***

<table>
<thead>
<tr>
<th>S.No</th>
<th>Constituents</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Quinones</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroids/Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Coumarins</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Phlobatannins</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Reducing sugar</td>
<td>+</td>
</tr>
</tbody>
</table>

+ indicates presence whereas – indicates absence

**Anti-inflammatory activity**

**Inhibition of Albumin denaturation method**

The inhibitory effect of different concentration of *Zea mays* on protein denaturation as shown in **Table.2. Zea mays** L. at a concentration range of 100, 200, 400, 800, 1600 µg /ml and standard 100, 200, 400, 800, 1600 µg /ml showed significant inhibition of denaturation of egg albumin in concentration dependent manner. Both membrane stabilization activity and effect on protein denaturation contribute to the *in vitro* anti inflammatory activity of *Zea mays* used in our study.

**Table 2: In Vitro Anti-Inflammatory activity of Ethanolic extract of Zeamays and Aspirin of Protein Denaturation Method**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th><em>Zea mays</em> ethanolic extract % inhibition</th>
<th>Aspirin standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>54.51±0.014</td>
<td>112.99±0.022</td>
</tr>
<tr>
<td>200</td>
<td>114.15±0.042</td>
<td>145.21±0.036</td>
</tr>
<tr>
<td>400</td>
<td>234.81±0.028</td>
<td>198.44±0.022</td>
</tr>
<tr>
<td>800</td>
<td>380.11±0.036</td>
<td>253.63±0.036</td>
</tr>
<tr>
<td>1000</td>
<td>578.40±0.223</td>
<td>320.29±0.036</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± Standard deviation

**HRBC Membrane Stabilization Method**

In the study of membrane stabilization activity of *Zea mays* L. at concentration range of 100, 200, 400, 800, 1000 µg /ml protected significantly in a concentration dependent manner
the erythrocyte membrane against lysis induced by hypotonic solution. Aspirin in the concentration of 100, 200, 400, 800, 1000 µg /ml used as standard also offered protection of HRBC membrane against damaging effect induced by hypotonic solution. The membrane stabilization action and inhibitory effect of different concentration of Zea mays is presented in Table. 3.

**Table 4: In Vitro Anti-Inflammatory Activity of ethanolic extract of Zea Mays And Aspirin of HRBC Membrane**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Zea mays ethanolic extract % inhibition</th>
<th>Aspirin standard % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>52.15±0.360</td>
<td>80.21±0.044</td>
</tr>
<tr>
<td>200</td>
<td>56.52±0.052</td>
<td>82.15±0.050</td>
</tr>
<tr>
<td>400</td>
<td>62.20±0.070</td>
<td>88.59±0.028</td>
</tr>
<tr>
<td>800</td>
<td>71.26±0.036</td>
<td>94.60±0.360</td>
</tr>
<tr>
<td>1600</td>
<td>74.39±0.142</td>
<td>96.48±0.028</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± Standard deviation

**DISCUSSION**

Protein denaturation is a process in which protein lose their tertiary structure and secondary structure by application of external stress or compound such as strong acid or base a concentration inorganic salt, an organic solvent or heat most biological protein lose their biological function when denatured. Denaturation of protein is a well documented cause of inflammation. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of plant extract to inhibit protein denaturation was studied.

Similarly, [13] reported that methanol extract of Murraya koenigii leaves produce significant Anti inflammatory activity in dose dependent manner in inhibition of protein denaturation. Padmanaban et al. (2012) [14] reported the inhibition of albumin denaturation of alcoholic extract showed significant inhibition of albumin denaturation.

Several anti-inflammatory drug have shown dose dependent ability to inhibit thermally induced protein denaturation [15]. Denaturation of protein is a well document cause of inflammation in condition like Rhumatoid arthritis. These protein against protein denaturation, which was the main mechanism of action of NSAIDs postulated by Mizushima and Kobayashi (1968) [16] before the discovery of their inhibitory effect of cyclooxygenase by Vane (1971) [17] may play an important role in the anti rheumatic activity of NSAIDs.

The HRBC membrane stabilization has been used as a method to study the in vitro anti-inflammatory activity because the erythrocyte membrane is analogues to the lysosomal membrane [18,19] and its stabilization implies that the extract may well stabilize the lysosomal membrane. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacteria enzyme and protease, which causes further tissue inflammation and damage upon extracellular release.

The lysosomal enzyme released during inflammation produce a various disorders. The extracellular activity of these enzymes are said to be related to acute to chronic inflammation. The non steroidal drugs act either by inhibiting the lysosomal enzymes or by stabilizing the lysosomal membranes [20].

The HRBC method was selected for the in vitro evaluation anti-inflammatory property because the erythrocyte membranes is analogues to the lysosomal membranes and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membranes is the important in limiting the inflammatory response by preventing the release of lysosomal constituents of activate neutrophil, such as bactericidal enzymes and protease, which causes further tissue inflammation and damage upon extracellular release [21].

**CONCLUSION**

The result of anti inflammatory studies showed that the inhibition of albumin denaturation at the highest concentration of 1000 µg / ml and membrane stabilization at the highest
concentration of 1600 µg/ml confirms the seed of *Zea mays* L. are potent inhibitor of acute and chronic inflammation.

**ACKNOWLEDGEMENT**

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**REFERENCES**

