In-vitro Anti-inflammatory and Anti-arthritic Activity of

Oryza sativa Var. Joha Rice (An Aromatic Indigenous Rice of Assam)

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Abstract: The objective of present work is to study the in-vitro anti-inflammatory and anti-arthritic activity of Joha Rice, an aromatic indigenous rice of Assam, India. The ethanolic extract of Oryza sativa Var. Joha rice (EEOS-JR) was studied for in-vitro anti-inflammatory activity by human red blood cell (HRBC) membrane stabilization method and In-Vitro anti-arthritic activity by bovine serum protein denaturation method and egg albumin denaturation method. The activity of ethanolic extract of Joha rice was compared with standard anti-inflammatory drug Diclofenac. In our result it is found that EEOS-JR at concentration of 100, 250 and 500 mcg/ml showed 51.12, 58.75 and 63.77% protection of HRBC in hypotonic solution respectively, whereas, standard diclofenac at 50, 100 and 250 mcg/ml which showed 68.11, 73.83 and 76.17% protection of HRBC in hypotonic solution respectively. It also showed 39.29%, 52.78% and 60.47% inhibition of denaturation @ 100, 250 and 500 mcg/ml of bovin serum whereas, standard diclofenac @ 100, 250 and 500 mcg/ml showed 93.20, 95.41 and 96.91% inhibition of denaturation of bovin serum. In egg albumin denaturation method at concentration of 100, 250 and 500 mcg/ml showed 75.00, 80.31 and 84.15% inhibition of egg albumin denaturation whereas, standard diclofenac @100, 250 and 500 mcg/ml which showed 27.78, 45.84 and 69.77% inhibition of egg albumin denaturation. It was found that ethanolic extract of Joha rice was more potent in inhibition of egg albumin denaturation than diclofenac. Finally, from results it can be concluded that Oryza sativa Var. Joha rice; an indigenous aromatic rice of Assam posses good in-vitro anti-inflammatory and anti-arthritic activities. By further extensive research, we can explore the medicinal value of joha rice.

Key words: Joha Rice • Aromatic Rice • In-vitro methods • Human red blood cell • Anti-inflammatory • Anti-arthritic activity

INTRODUCTION

Assam is a land of thousands of natural herbs and medicinal plants. The unique geographical location, abundant of fertile soil, friendly climate and high rain fall make a gift of herbal resources. With its vast hills and forests, Assam is the home to a variety of medicinal plants such as word famous Tea (Camelia sinensis), Sarpagandha (Rauvolfia serpentine Benth.ex.Kur), Pippali (Piper longam Linn), Amlaki (Emblica officinalis Gaertn), Hilikha (Terinalia chebula Retz.), Bhomora (Terminalia beleraica) etc. More than 300 medicinal plants have been identified in Assam but only about 5-10% of the plants and herbs are currently utilized [1] and the rest hold a vast potential. Lack of knowledge, space and adequate facilities are playing important hurdles to save the unique gift of nature in Assam for making scientifically evident medicinal drug in present market. Joha Rice is one of the 40,000 varieties of species Oryza sativa and it is popular for its great aroma and equally remarkable taste. It is commonly used to prepare Khir or Payash or Pulaol like traditional recipe [2].

Some literature available which mentioned the antioxidant properties of rice. Laokulidilok et al. reported that several pigmented rice brans have free radical scavenging and antioxidant activity [3]. Muntana and Prasong reported total phenolic contents and their antioxidant activities of Thai white, red and black rice bran extracts [4]. Rao et al. reported antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran [5].

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Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain [6]. Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, hey fever, ischemic heart diseases [7, 8, 9] etc and inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vascularitis, celiac diseases, auto-immune diseases etc [7, 8, 9].

Anti-inflammatory drugs like NSAIDs used to reduce the swelling and pain of inflammation. But these agents carry the risk of gastro-intestinal toxicity, cardiovascular and other toxicity for prolonged use [11]. For these reason, there is a need for anti-inflammatory drugs having less severe side effects to use for chronic inflammatory disease as well. Therefore, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is a lack of proper scientific evidence.

The objective of present work is to study the in-vitro anti-inflammatory and anti-arthritic activity of *Oryza sativa* (var. Joha Rice): An aromatic indigenous rice of Assam, India.

**MATERIALS AND METHODS**

**Chemicals and Instruments:** Diclofenac (Symed Pharm. Pvt. Ltd, Hyderabad), all other reagents used were of analytical grade. Instruments UV/VIS Spectrophotometer (LABINDIA, UV 3000+), Microcentrifuge (REMI, RM-12 C)

**Plant Material and Extraction Procedures:** *Oryza sativa* (var. Joha Rice) was collected from local cultivators of Assam and was authenticated by Prof. Dr. K. Madhava Chetty, Taxonomist, SVU University, Chithoor andhra Pradesh (India). Joha Rice was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. This powder was packed into soxhelet apparatus and extracted successively with ethanol.

**Preliminary Phytochemical Analysis:** The ethanolic extract of Joha Rice was subjected to preliminary phytochemical screening using standard methods [12].

**In-vitro Anti-inflammatory Activity by Human RBCs Membrane Stabilization Method:** The human red blood cell membrane stabilization method was used for study of anti-inflammatory activity [13]. The blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared viz., 100, 250 and 500 mcg/ml using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It is incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. The haemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm.

**Bovine Serum Protein Denaturation Method**

**Preparation of Reagents**

**0.5% Bovine Serum Albumin (BSA):** Dissolved 500mg of BSA in 100 ml of water.

**Phosphate Buffer Saline PH 6.3:** Dissolved 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na HPO ), 0.24 g of potassium dihydrogen phosphate (KHPO ) in 800 ml distilled water. The pH was adjusted to 6.3 using 1N HCl and make up the volume to 1000 ml with distilled water.

**Method:** Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5%W/V aqueous solution) and 0.05ml of test solution of various concentrations.

Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (0.5%W/V aqueous solution) and 0.05ml of distilled water.

Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution.

Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5%w/v aqueous solution) and 0.05ml of Diclofenac sodium of various concentrations.

**Procedure:** 0.05 ml various concentrations (50, 100, 250 ig/ml) of test dugs and standard drug diclofenac sodium (50, 100, 250 ig/ml) were taken respectively and 0.45 ml (0.5% w/V BSA) mixed. The samples were incubated at...
37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 255 nm. The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. The percentage inhibition of protein denaturation can be calculated as:

\[
\text{Percentage Inhibition} = 100 - \left(\frac{(\text{optical density of test solution} - \text{optical Density of control})}{\text{optical density of test}}\right) \times 100
\]

**Egg Albumin Denaturation Method:** The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations (100, 250, 500 μg/mL) of drug. A similar volume of double-distilled water served as the control. Next, the mixtures were incubated at 37 ± 2°C in a BOD incubator for 15 minutes and then heated at 70°C for five minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Diclofenac sodium in the concentrations of 100, 250, 500 μg/mL was used as the reference drug and treated similarly for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

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

Where,

\[V_t = \text{absorbance of the test sample,}\]
\[V_c = \text{absorbance of control.}\]

Each experiment was done in triplicate and the average was taken.

The extract concentration for 50% inhibition (IC 50) was determined by the dose-response curve.

### RESULTS

The Joha rice was extracted with ethanol and an oily semisolid brownish black colour extract was found. The percentage yield was 3.6% (w/w).

The ethanolic extract of Joha rice was found to contain different phytoconstituents like proteins, terpenoids, phenolic compounds, flavonoids, carbohydrates and volatile oils.

**In-vitro Anti-Inflammatory Activity by HRBC Method:**

In *in-vitro* anti-inflammatory activity, EEOS-JR at concentration of 100, 250 and 500 mcg/ml showed 51.12, 58.75 and 63.77% protection of HRBC in hypotonic solution respectively, whereas, standard diclofenac at 50, 100 and 250 mcg/ml which showed 68.11, 73.83 and 76.17% protection of HRBC in hypotonic solution respectively (Table 1 and Fig. 2).

**In-vitro Anti-arthritis Activity by Bovine Serum Denaturation Method:**

In *in-vitro* anti-arthritis activity by Bovine Serum denaturation method at concentration of

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![Fig. 1: Ethanolic extract of oryza sativa (var. Joha Rice) (EEOS-JR).](image-url)
Table 2: Effect of EEOS-JR in *in-vitro* anti-arthritic activity on bovine serum protein denaturation method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µg/ml)</th>
<th>Test Absorbance</th>
<th>Product Control</th>
<th>% Denaturation</th>
<th>% Inhibition of denaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.029</td>
<td>0.012</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>EEOS-JR</td>
<td>100</td>
<td>0.047</td>
<td>0.019</td>
<td>60.71</td>
<td>39.29</td>
</tr>
<tr>
<td>EEOS-JR</td>
<td>250</td>
<td>0.054</td>
<td>0.018</td>
<td>47.22</td>
<td>52.78</td>
</tr>
<tr>
<td>EEOS-JR</td>
<td>500</td>
<td>0.064</td>
<td>0.021</td>
<td>39.53</td>
<td>60.47</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>100</td>
<td>0.272</td>
<td>0.022</td>
<td>6.80</td>
<td>93.20</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>250</td>
<td>0.412</td>
<td>0.036</td>
<td>4.59</td>
<td>95.41</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>500</td>
<td>0.607</td>
<td>0.052</td>
<td>3.09</td>
<td>96.91</td>
</tr>
</tbody>
</table>

Fig. 2: % inhibition Vs Concentration (Linear analysis of *in-vitro* anti-inflammatory activity EEOS-JR and Diclofenac Sod.).

Fig. 3: % inhibition VS Concentration *in-vitro* anti-arthritic activity (EEOS-JR and Diclofenac Sod.) on bovine serum protein denaturation method.
Fig. 4: % inhibition VS Concentration in-vitro anti-arthritic activity (EEOS-JR and Diclofenec Sod.) on egg albumin denaturation method.

Table 3: Effect on protein denaturation (Fresh egg albumin).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (µg/ml)</th>
<th>Test Absorbance</th>
<th>Product Control</th>
<th>Percentage Denaturation (%)</th>
<th>Percentage of inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.024</td>
<td>0.011</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>EEOS_JR</td>
<td>100</td>
<td>0.064</td>
<td>0.012</td>
<td>25.0</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0.082</td>
<td>0.016</td>
<td>19.69</td>
<td>80.31</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.096</td>
<td>0.014</td>
<td>15.85</td>
<td>84.15</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>100</td>
<td>0.030</td>
<td>0.012</td>
<td>72.22</td>
<td>27.78</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0.044</td>
<td>0.020</td>
<td>54.16</td>
<td>45.84</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.066</td>
<td>0.023</td>
<td>30.23</td>
<td>69.77</td>
</tr>
</tbody>
</table>

100, 250 and 500 mcg/ml showed 39.29, 52.78 and 60.47% inhibition of denaturation of bovin serum whereas, standard dicrofenac at 100, 250 and 500 mcg/ml which showed 93.20, 95.41 and 96.91% inhibition of denaturation of bovine serum (Table 2 and Fig. 3).

**DISCUSSION**

The Ethanolic extract of *Oryza sativa* Var Joha Rice (EEOS-JR) tested for phytochemical constitutents like reducing sugars, phenolic compounds, flavanoids, protein, carbohydrates and volatile oils. The knowledge of the chemical constituents of plants helps to screen for biological activities [17]. The phenolic and flavanoids are widely distributed secondary metabolites in plants having anti-oxidant activity [18, 19, 20, 21] and have wide range of biological activities as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [22].

The ethanolic extract of *Oryza sativa* (var. Joha Rice) subjected to preliminary phytochemical screening using standard laboratory and also screened for physical properties and found that EEJR was in brownish black colour, insoluble in water and soluble in all organic solvents. The phytochemical tests confirm the presence of carbohydrates, terpenoids, glycosides, proteins, phenols, saponins, alkaloids, flavonoids and tannins.

Inflammation is the tissue response to injury and involves a complex process of enzymatic reactions [23]. The vitality of cells depends on the integrity of their membrane. If Red Blood Cells (RBC’s) exposed to injurious substances such as hypotonic medium then lysis of its membrane will occur accompanied by haemolysis and oxidation of haemoglobin. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such type of injury to RBC membrane will cause secondary damage through free radical induced lipid peroxidation [24, 25]. It is therefore expected that compounds with membrane-stabilizing properties, should offer significant protection of cell membrane against injurious substances [26, 27, 28]. Compounds with membrane-stabilizing properties are well known for their ability to interfere with the release of phospholipases that trigger the formation of inflammatory mediators [29]. In our study, HRBC membrane stabilization method, absorbance of Hemoglobin is taken. The hemoglobin is released as a result of lyses of RBC membrane. Due to stabilization of membrane less absorbance is noted in spectrometer results. The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. EEOS-JR has shown significant membrane stabilizing property, which suggests that its anti-inflammatory activity observed in this study, may be related to the inhibition of the release of phospholipases that trigger the formation of inflammatory mediators. RA, the common human autoimmune disease is characterized by chronic inflammation in joints followed by pannus formation with infiltrated lymphocytes and fibrinoid joints of synovial membrane with concomitant destruction of cartilage and bone.

Some literature reported that denaturation of protein is one of the cause of rheumatoid arthritis [23, 30]. Production of auto-antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation [31]. In our present study, ethanolic extract of *Oryza sativa* (var. Joha Rice) inhibited heat induced protein denaturation and may be one of the reason of possessing anti-inflammatory and anti-arthritic activity.

**CONCLUSIONS**

Finally, it can be concluded that Joha rice—an indigenous aromatic rice of Assam posses good *in-vitro* anti-inflammatory and anti-arthritic activities. By further extensive research, we can explore the medicinal value of joha rice and make reasons why this is used traditionally for various diseases.

**REFERENCES**