Effect Of *Opuntia Ficus indica* On Diclofenac Sodium Induced Gastric Ulcer On Rats

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**ABSTRACT**

Prolong intake of DFSOD induces a gastric ulcer, 55-65% of the world’s population suffers from different kind of ulcers. *Opuntia ficus indica* is the family of plant genus cactus Cactaceae, the common name is prickly pears. The present study is designed as effect of *opuntia Ficus indica* on diclofenac sodium induced gastric ulcer on rats. Characterization of OFIAQE extract by TLC method, Evaluation of anti-oxidant property was performed by using DPPH and Hydroxyl radical scavenging assay. Evaluation of ulcer genic activity by calculating ulcer index and histopathology. Rf values of OFIAQE 0.9 whereas, standard is 1, % inhibition of OFIAQE is showed similar to the standard i.e., ascorbic acid. DFSOD has shown significant difference when compared to the normal i.e. 12.88±1.02, whereas OFIAQE treated group has shown ameliorative effect when compared with positive control and normal group i.e. 6.12± 0.98 and positive control show near to the normal i.e. 3.02± 1.17. DFSOD treated group has shown the Section severe disruption of the epithelial surface and submucosal layer edema with infiltration of leukocytes in diclofenac sodium 10mg/kg-treated rats, rats treated with OFIAQE 400 mg/kg repaired serosa and subsersal layers, whereas, treated with postive control 5mg/kg showing normal histopathology. However, more extensive studies are required to confirm the effect of OFIAQE usage for identifying its mechanism of action of anti-ulcer activity.

**Keywords:**
Anti-Ulcer, Diclofenac Sodium, Opuntia Ficus Indicia And Gastric Ulcer

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INTRODUCTION:
Nonsteroidal anti-inflammatory drugs (NSAIDs), such as diclofenac sodium, are is used as a chronic administration for the treatment of rheumatoid arthritis. Prolong intake of DFSOD induces a gastric ulcer, 55-65% of the world’s population suffers from different kind of ulcers [1]. An ulcer is identified by the distraction of mucosal veracity due to active inflammation that prominents to local defects. Diclofenac sodium is commonly prescribed for antipyretic, analgesic and anti-inflammatory properties; its action is mediated by inhibition of the prostaglandin biosynthesis, cyclooxygenase, and leukotriene [2]. Diclofenac sodium induces gastric mucosal lesions because it is highly acidic in nature and gastric environment favors the migration of nonionized lipophilic diclofenac sodium into the epithelial cells, which are dissociated into ions on the surface, trapping hydrogen ions and causing mucosal damage [3]. This action is further enhanced by the reduction of the following: Mucosal blood flow, mucous and bicarbonates secretion, and gastric defensive factors. Oxidative stress in mucosal cells, another etipathogenic factor that induces a gastric ulcer is convoluted [4].

All these factors cause an imbalance between the ejection of acid pepsin secretion and defensive factors, including secretion of mucin and shedding of cells. The effect of diclofenac can be reduced by the apposite use of antioxidants that ameliorate the free radical [5]. Plants possess valuable phytochemicals in the form of secondary metabolites, the antioxidant properties of which are flavonoids and phenolics. Studies on antioxidants have recived considerable attention in recent years, as these chemicals can help to protect the biological systems against diseases and injuries [6].

Histamine (H2) receptor antagonists, proton pump inhibitors, antacids, and anticholinergics are the traditional drugs used in the treatment of gastric ulcers [7]. Most of these medicines have severe side effects and interaction with drugs. Alternative and complementary drug systems may help to reduce the side effects, however, provide additional gastric damage therapeutics [8]. Opuntia ficus indica is the family of plant genus cactus Cactaceae, the common name is prickly pears, It is grown for medical purposes, and are native to Europe, Mediterranean countries. Prickly pears fruits and leaves are edible. It is widely used for the treatment of anti-inflammatory and, it has an anti-oxidant property [9]. Therefore, the present research work designed as an effect of opuntia ficus indica on diclofenac sodium induced gastric ulcer in rats.

MATERIALS AND METHODS

Plant material

Opuntia ficus indica (OFI) fruit was collected in and around Nellore rural area of Andhra Pradesh, 2015. Botanically identified and authenticated by Dr. J. SURESH, Assistant Professor, Dept. of Pharmacognosy, JSS College of Pharmacy, Mysore. The fruit was cut into two equal halves and kept for air dried i.e 28 days, mechanical grinding and processing powder was prepared, which was used for further studies.

Animals

The experiments were carried out on adult Wistar Albino rats weighing 120-150 gms of either sex. They were obtained from the JSS Medical College Central Animal House Facility in Mysore. In controlled temperature and humidity conditions, animals were kept in sterile polypropylene cages containing sterile paddy husk as bedding material with a maximum of six animals in each cage. The rats were fed with standard food pellets and water ad libitum. The studies were carried out were approved by Institutional Animal Ethical Committee, JSS College of Pharmacy, Mysore, Karnataka. Approval no: 178/2015

Preparation of OFI fruit extract

Opuntia ficus-indica (Prickly pear) was collected. Spines were removed from the prickly pear and it was cut into two half to prevent loss of active constituents and it was kept in a hot air oven for 2 days at 32° c to 35° c to eliminate water continent from it further it was taken for shade dry [10]. The extract was prepared by cold maceration process. The dried fruit powder of OFI was passed through sieve 22# and about 766.37 gm. The powder was taken in a round bottom flask, water and 5% of alcohol were added. The flask was
shaken frequently and the solvent was changed at every 3 days of interval up to 12 days. The filtrate was concentrated in a hot water bath and the extract was collected. That was aqueous extract and named as OFIAQE (*Opuntia ficus indica* aqueous extract). The extract was calculated for percentage yield and stored in desiccators [11].

**Thin layer chromatography**
Powdered drug (1g) is extracted by heating under reflux for 10 minutes with 10ml of water. The filtrate is evaporated to 3ml, and 20µl is used for TLC. Methanol: water (3:3) was used as a solvent system. The TLC chamber is allowed to saturate with a solvent system for half an hour. 1mg standard compound (β-sitosterol) was dissolved in 1ml methanol; 10µ was used for TLC. Silica gel plates were spotted with standard and samples and developed in the solvent system. Spots were visualized under UV chamber. Noted the distance travelled by sample and Rf values were calculated.

**DPPH scavenging activity**
The extract antiradical activity was estimated as described by Olugbami et.al.[12]. The absorbance (A) was measured at 518 nm using a UV/VIS spectrometer. The percentage of radical scavenging activity (RSA) was calculated based on the following equation:

\[
\text{DPPH Scavenged} \% = \frac{(A_{\text{cont}} - A_{\text{sample}})}{A_{\text{cont}}} \times 100
\]

\(A_{\text{cont}}\) and \(A_{\text{sample}}\) are the absorbance values (at 518 nm) for the control and sample, respectively.

**Hydroxyl radical scavenging assay**
Hydroxyl radical scavenging activity was measured by the ability of the extract to scavenge the hydroxyl radicals generated by the Fe³⁺-ascorbate-EDTA-H₂O₂ system described as Halliwell et.al. [13]. The scavenging activity on hydroxyl radicals was expressed as

\[
\text{Inhibition} \% = (1 - \frac{A}{A_0}) \times 100
\]

where \(A_0\) is the absorbance of the negative control (without sample) at 532 nm, and \(A\) is the absorbance at 532 nm of the reaction mixture containing the sample.

**Induction of ulcergencity in the rat model**
The ulcer was induced to rats by using Diclofenace sodium 10 mg/kg, p.o for 28 days[14].

**Experimental protocol**
In this study, the experiments were designed with four groups of rats (n = 6). Group-I was used as a normal control group. Group-II untreated (control) and had free access to food materials. Group-III was subjected OFIAQE ( 400 mg/kg, p.o for 28 consecutive days) Group IV was subjected to positive control i.e. Ranitidine (5 mg/kg, p.o. for 28 days). On 28th day animals were sacrificed and collected stomach samples for ulcer index and histopathology.

**Ulcerogetic activity**
Stomachs were isolated and opened along a higher curvature to expose the inner surface at the end of the study for ulcerogeticity screening. The inner surface was thoroughly washed with normal saline and observer with the naked eye for any damage to the gastric mucosa, bulging and inflammation of each stomach injury [15].

\[
\text{Ulcer index} = \frac{\text{ulcerated area} + \text{total stomach area}}{\text{total stomach area}} \times 100
\]

**Histopathological assessment**
At the end of the experiment, all animals were sacrificed. The animals stomach from each group was isolated and set for histopathology analysis in 10 percent formalin. The tissues were sectioned at 6.0 µm and stained with haematoxylin and eosin [16].

**Statistical analysis**
All the results were expressed as the mean ± standard deviation (SD). Data obtained from all behavior tests and tissue biomarkers were statistically analyzed using one-way analysis of variance (ANOVA). Further, Tukey’s test was applied for Post-hoc analysis using Graph pad prism Version-5.0 software. A probability value of \(p < 0.05\) was considered to be statistically significant.

**RESULTS**
**Characterization of OFIAQE extract by TLC method**
The observation from TLC data shows that the proportion of mobile phase (methanol : water) shows...
good separation of components with the Rf value and Beta sitosterol. It is shown in Table 1 and Figure 1.

**Diphenyl picrylhydrazyl (DPPH) free radical scavenging assay**

The Percentage free radical scavenging activity of OFIAQE extracts using the DPPH method. OFIAQE moderately scavenged DPPH radicals, whereas the ascorbic acid used as a reference standard. Percentage of inhibition was shown in Figure 2.

**Hydroxyl radical scavenging assay**

Free radical scavenging activity of OFIAQE extracts by hydroxyl radical scavenging assay. OFIAQE moderately scavenged hydroxyl radicals respectively whereas the ascorbic acid used as a reference standard showed scavenging activity. The different concentrations were of free radical activity of OFIAQE is shown in Figure 3.

**Ulcerogenic activity**

Stomach was isolated and observed inner curvature of the stomach, more redness was found in the negative control group. When compared with all other groups. Ulcer index of OFIAQE 400 mg/kg has shown significant when compared with the negative control group whereas positive control has shown an ameliorative effect when compared to the normal group. The results were expressed in Table 2.

**Histopathology**

The observed histopathological changes in necrosis of the surface epithelium and damage of the tissue in the negative control group when compared with normal. The treatment of OFIAQE 400 mg/kg shown decreased in the necrosis, inflammation, and damage of the tissue when compared with alone negative control group. The results were expressed in Figure 4.

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**Table 1: Characterization of OFIAQE extract by TLC method**

<table>
<thead>
<tr>
<th>Components</th>
<th>Rf values(cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta sito-sterol</td>
<td>1</td>
</tr>
<tr>
<td>OFIAQE</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**Figure 1: OFIAQE extract by TLC method**
Figure 2: Percentage of inhibition of ascorbic acid and OFIAQE by DPPH radical scavenging assay.

![Graph showing percentage inhibition of ascorbic acid and OFIAQE by DPPH radical scavenging assay.]

Figure 3: Percentage of inhibition of ascorbic acid and OFIAQE extract by hydroxyl radical scavenging assay.

![Graph showing percentage inhibition of ascorbic acid and OFIAQE by hydroxyl radical scavenging assay.]

Table 2: Effect of OFIAQE on DFSOD induced ulcer

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.64±1.99</td>
</tr>
<tr>
<td>Negative Control (10)</td>
<td>12.88±1.02</td>
</tr>
<tr>
<td>OFIAQE (400)</td>
<td>6.12± 0.98</td>
</tr>
<tr>
<td>Positive control (5)</td>
<td>3.02± 1.17</td>
</tr>
</tbody>
</table>

Parenthesis numbers indicate mg/kg dose. Data were expressed as mean ± SD, n = 6, one way Analysis of Variance (ANOVA) followed by multiple comparisons of the Tukey’s test; *p < 0.05 Vs normal group. *p < 0.05 Vs control group. Abbreviation: DFSOD; diclofenac sodium and QFAQE; opuntia ficus indica aqueous extract.
Figure 5: Descriptive images showing morphological changes in rat gastric tissues after administration of diclofenac, OFIAQE and ranitidine.

Figure 6: Descriptive images showing histological observations in rat gastric tissues after administration of diclofenac sodium, as well as OFIAQE and positive control.

DISCUSSION

Inhibitory action of NSAIDS on prostaglandin amalgamation coupled with free radicals formation has been preached as critical biochemical events with in the pathogenesis of gastric ulceration [17]. It may be relevant to understand these events could be of relevance in the design of new anti-ulcer drugs [18]. With the inherent side effects impacts and the impressively high cost of synthetic drugs, the most appropriate treatment for gastric ulcers by exploiting natural products from plant sources, which are believed to be non-toxic, effective and affordable. Phototherapy is gaining rapidly in the maintenance of human health and in the prevention of certain diseases.
such as gastric ulcer which causes by drug toxicity [19]. This is due to the possession of phytonutrients with excellent antioxidant properties that play a major role in toxicity related disorders. Negative Control group i.e. DFSOD showed significant to the normal group. Whereas OFIAQE (400 mg/kg) shown significant activity via regulation of free radical generations, lipid peroxidation process. These effects are comparable with negative control.

Hence, it may be concluded that the administration of OFIAQE can be used to reduce the gastric ulcer induced by DFSOD. OFI fruit contains flavonoids, polyphenols, volatile oil, and vitamins constituents which may be responsible for the anti-inflammatory, anti-arthritis and anti-ulcer activity [20]. However, more extensive studies are required to confirm the effect of OFIAQE usage for identifying its mechanism of action of anti-ulcer activity.

ACKNOWLEDGMENT

The authors are thankful to the JSS Academy of Higher Education and Research, JSS College of Pharmacy, Mysuru -570 015, Karnataka (India) for their unconditional support and for providing technical facilities to carry out this research work.

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