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Research Article

ANTIOXIDANT AND ANTI INFLAMMATORY PROPERTIES OF *FLEMINGIA WIGHTIANA* ETHANOLIC EXTRACT

M. Lakshmi Priya[1], C. Varadarajulu Naidu[2], and Josthna Penchalaneni[1*]

[1]*Department of Biotechnology, Sri PadmavathiMahila University, Tirupati, Andhra Pradesh, India*

[2]*Department of Biotechnology, Dravidian University, Kuppam, Andhra Pradesh, India*

**Corresponding author: penchalajyo@yahoo.co.in*

ABSTRACT

In the legume family Fabaceae, *Flemingia* is a genus of endemic flowering plants with medicinal values. The leaf extract of several species of *Flemingia* is traditionally used in Asia, primarily in India and China, for the treatment of various diseases. However, chemical and biological properties and activities of *Flemingia* species are poorly investigated. In this study, we have assessed the antioxidant and anti-inflammatory properties of *Flemingia wightiana* ethanolic extract (FWEE) in rat model. The study includes the estimation of the antioxidant role of FWEE by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2) methods. The further study proceeds towards the estimation of role of FWEE in the dextran sulfate sodium (DSS) induced Colitis model by observing weight loss, colonic shortening, histological damage and levels of cyclooxygenase 2 (COX-2). Obtained results revealed that the FWEE had appreciable anti-oxidative and anti-inflammatory activities and thus justifies its use in traditional medicine in the treatment of inflammatory diseases and other disease conditions.

Keywords: *Flemingiawightiana*, Flavonoids, phenols, DSS, Inflammation, COX-2

1. INTRODUCTION

Of various ailments ranging from the common cold to cancer, plant-based drugs have been used since time immemorial. Traditional herbal medicine in developing countries is an important component of primary health care systems [1]. Medicinal plants play an important role in many peoples' daily lives and are an important part of the Indian cultural heritage [2].In addition, the increase in population, insufficient drug supply, prohibitive treatment costs, side effects of several synthetic drugs and the growth of resistance to currently available drugs for many diseases have resulted in increased focus on the use of plant materials as a source of medicinal products for a wide variety of human diseases [3,4]. Even the World Health Organization (WHO) has estimated that 80% of the world's inhabitants use traditional medicine for their primary health care needs, and much of this therapy requires the use of herbal extracts and their active ingredients [5].Therefore, in the world, more than 20,000 herbal plants are known to be used for medicinal purposes [6].Among the various secondary metabolites, flavonoids and other phenolic compounds, which hold at least one hydroxyl group of aromatic rings, are well known for their medicinal relevance [7].

Such phytochemicals have been found to be active antioxidants, anti-cancer, antibacterial, cardioprotective agents, anti-inflammation, the enhancement of the immune system, the defense of the skin from UV radiation, and an important candidate for pharmaceutical and medical usage [8,9]. Phytochemicals also exhibit antioxidant activity, and it has been documented that *Flemingia* species possess several phytochemicals, including flavonoids and phenols [10, 11]. The aim of this study is to examine the curative effect of *Flemingia wightiana* against colitis, which refers to inflammation of the colon's inner lining. *F. wightiana* belongs to the medicinally important family of Fabaceae, which has been less studied and may have a potential impact on colitis management due to the existence of active phytocompounds.

2. MATERIAL AND METHODS

2.1. Preparation of plant material

The leaves were initially washed in tap water, followed by distilled water. Then the leaves were dry-shaded and crushed at 60°C by a Soxhlet extractor to powder and subjected to extraction. The extraction was done with ethanol and filtered to yield the extract. To achieve a

solid mass, the extract was concentrated to dryness and used for further tests.

2.2. Total Phenolic Content

Following the method described by Kamtekar et al [12] the total extract phenolics were calculated using the Folin and Ciocalteu reagents. In brief, gallic acid was used to make the standard calibration curve.Specific gallic acid concentrations (10, 20, 40, 60, 80, 100 µg / ml) and test samples (1mg / ml) consisting of 1mL of methanol are combined with 0.5mL of Folin-Ciocalteu's phenol reagent (1:1). After 5 minutes, the mixture was added 1.5mL of saturated sodium carbonate solution (8% w / v in liquid) and the volume of distilled water was made up to 10mL. The reaction was kept in the dark for 2h and measured at 750 nm. Based on the standard curve of gallic acid, the phenolic content was calculated as GAE / g equivalents of dry plant material. All determinations were performed in triplicates.

2.3. Total Flavonoids Content

The colorimetric method of aluminum chloride was used to assess the plant extract's total flavonoid content. Quercetin was used to make the standard calibration curve for the complete determination of flavonoid. Different concentrations of quercitin (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg /ml) and test sample (1 mg/ml) made up to 1mL with ethanol was mixed with 4 mL distilled water. 0.3mL of 5% Sodium nitrite was added to all tubes and after 5 min, 0.3mL of 10% Aluminium chloride was added. 1 M Sodium hydroxide was then added to all tubes and volume was made up to 10 mL with distilled water. After mixing, the solution was incubated for 30min at room temperature. The absorbance of the reaction mixtures was measured against blank at 510nm wavelength with a UV-Vis spectrophotometer (Labman UV-Vis Spectrophotometer). The concentration of total flavonoid content in the test samples was calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of dry plant material. All the determinations were carried out in triplicates.

2.4. Evaluation of Antioxidant activity

To evaluate the antioxidant activity of FWEE, we have performed both DPPH photometric assay and hydrogen peroxide scavenging activity

2.5. DPPH photometric assay

DPPH photometric assay was performed as per the method developed by Zhishen et al., [13] In brief, themethanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$\% \text{ Scavenging activity} = \frac{\text{A Control - A Sample}}{\text{A Control}} \text{ X } 100$$

Where A control is the absorbance of DPPH radical+ methanol; A sample is the absorbance of DPPH radical+ sample extract.

2.6. Hydrogen peroxide scavenging activity

The Hydrogen peroxide-scavenging activity of extract was determined by the method of Ruch et al., [14]. The extract was dissolved in 3.4 mL of 0.1M phosphate buffer (pH 7.4) and mixed with 600 µL of 43 mM solution of hydrogen peroxide. The absorbance value (at 230 nm) of the reaction mixture was recorded at 10 min intervals between zero and 40 min.

Percentage scavenged [H_2O_2] = 1- Abs (standard)/Abs (control) x100

Where control is the absorbance of H_2O_2; standard is the absorbance of H_2O_2 + sample extract.

2.7. Dextran sulfate sodium (DSS) colitis model – FWEE treatment

Animal experiments were carried out in accordance with the principles of the animal ethics committee and approved by the committee (Reg. No. 1077/PO/a/12/CPCSEA/ SPMVVIEC/2014/02). Male Wistar rats with 140-160 g weights were used for this study. Rats were maintained in plastic cages enclosed in a wire mesh. Temperature was maintained at 24°C and a humidity of 55+5 % under a 12h light-dark cycle.

In this study, the most commonly used DSS was used to induce epithelial damage, colitis to monitor the performance of FWEE treatment. All the mice were fed with DSS for 6 days for the DSS mouse model (whereas the DSS group consisted of 5 percent DSS in drinking for 6 days). Rats were randomly divided into four groups (n=5) as follows;

Group-1 stands as control received distilled water

Group-2 received FWEE (300mg/kg)

Group-3 received the standard drug, 5 % DSS

Group-4 received the both 5% DSS and FWEE (300mg/kg).

To determine whether FWEE can treat colitis, mice colon tissues were collected by which were then washed with PBS (phosphate-buffered saline), cut longitudinally, swiss-roled, then formalin fixed and paraffin embedded for further studies including histology.

2.8. Histopathological examination

For histological examination, a sample of colonic tissue located 4-5 cm above the anal margin was obtained from rats in all treatment groups. The sections were stained with hematoxylin and eosin using routine techniques. Histological colonic damage was scored according to Sykes criteria [15].

2.9. Statistical analysis

In triplicate, all measurements were performed and the results were represented as mean ± SEM. Using one-way ANOVA, statistical data analyzes were achieved and the significance level was set at $P< 0.05$.

3. RESULTS AND DISCUSSION

3.1. Total phenolic and flavonoid content

The results of our preliminary phytochemical analysis revealed that the aqueous extract of FWEE contained both flavonoids and phenols. As per the results of the aluminium chloride colorimetric test, the total flavonoids in the FWEE is 18.175 ± 0.117 mg QE/g dry weight of extract. In addition, as per the results of Folin and Ciocalteu reagent colorimetric test, the total phenolic content in the FWEE is 92.468 mg QE/g dry weight of extract. As the abundant phenol and flavonoid content is present in FWEE, then the FWEE may consider as indispensable component for biomedical applications with a broad spectrum of health promoting effects.

3.2. Evaluation of Antioxidant activity

As antioxidants have become a subject of increasing interest to researchers in health and food sciences, we have estimated the percentage of FWEE's free radical scavenging activity for DPPH and H_2O_2 and the findings are presented in **Table 1.** The ethanolic extract of exhibited a maximum DPPH scavenging activity of 83.83% at 400 µg/ml; whereas for H_2O_2, it shows 85.43% at 400 µg/ml. Medicinal plants have long been documented as a prospective hub of natural antioxidant compounds, particularly plant secondary metabolites, *i.e.,* plant-generated phenolic compounds and flavonoids to protect themselves or promote growth under adverse conditions.

Table 1: Percentage of scavenging activity by DPPH and H_2O_2

Concentration (µg/ml)	% scavenging activity by DPPH	% scavenging activity by H_2O_2
50	35.31/±1.22	37.19/±1.85
100	48.33/±1.2	47.39/±0.78
200	63.0/±0.77	57.5/± 0.95
300	71.87/±1.45	68.96/±0.67
400	83.83/±1.31	85.43/±2.0

Furthermore, functional group arrangement, configuration, substitution, number of hydroxyl groups were also influenced by flavonoid antioxidant activity, such as radical scavenging activity and/or metal ion chelation capability. Based on the results of this study FWEE reveals abundant quantities of phenolic compounds as well as flavonoids, well-known as antioxidants, and therefore FWEE could support human health, cure and prevent many diseases. In addition to these advantages, antioxidants also contribute significantly to the food industry.

3.3. Changes in body weights

Evaluation of body weights is one of the supportive parameter in inflammatory studies. Therefore, in this study, after administration of FWEE (300mg/kg) over six days we have assessed the impact on the rat body weight and the results are summarized in Figure 1.

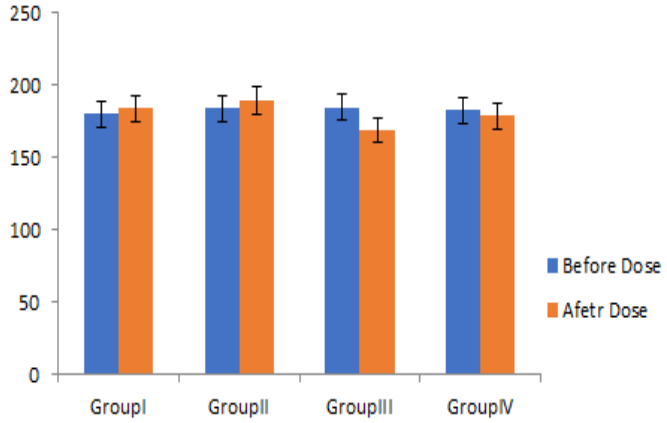

Fig. 1: Changes in body weights of the rats before and after induction of DSS

As per the obtained results, body weights of control group rats are remains same throughout the experimental period. A slight increase and an abnormal decrease in body weight have seen in FWEE treated and DSS treated groups respectively. Significant inflammation recovery impression is imparted through the recovery in body weights of group IV which is further confirmed by histology and immunohistochemistry results.

3.4. Study of colon length changes

Colon lengths were measured upon sacrificing the rats. A comparative study of colon length in different groups reveals that colon length shrinks due to stress and inflammation in DSS treated group. The control group possesses a colon length of 17.9 cm. The however slight increase in colon length in FWEE treated group (18.3) was observed (Figure 2).

In this study, group-3, which received the standard drug, 5 % DSS have shown a reduction in their length. Group 2, which received FWEE (300mg/kg) have shown higher colon length and group 3, which received the standard drug, 5% DSS has shown significantly reduced colon length. Group-4, which received both 5% DSS and FWEE (300mg/kg) have shown slightly increased colon length than the group. The obtained results confirm the influence of FWEE.

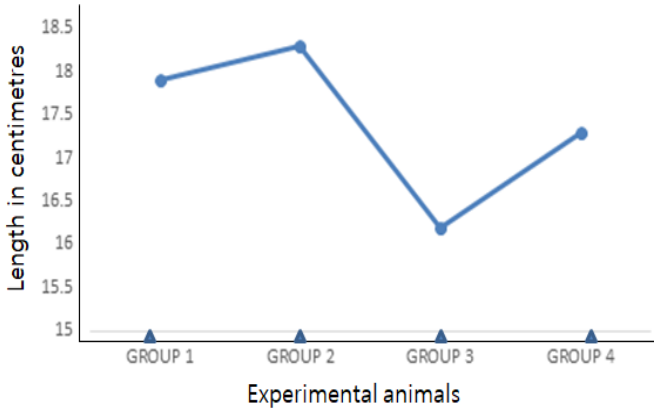

Fig. 2: Representing colon lengths in different groups at the end of experiment

3.5. Histological analysis

Effects of FWEE on the prevention of inflammation and ulceration in the colon of different experimental groups are presented in Figure 3. The histopathological studies state that normal colon structure was observed in the slide of control group I. Plant extract-treated group II shows similar histology results as a control group with normal mucosal layer. Group III mice were shown wide areas of mucosal necrosis with cytological and nuclear atypia including loss of differentiation (goblet cells) and enlarged nuclei. Group IV which is DSS and FWEE treated group have shown a considerable mucosal damage recovery. The obtained results confirm the effective role of FWEE on inflammation.

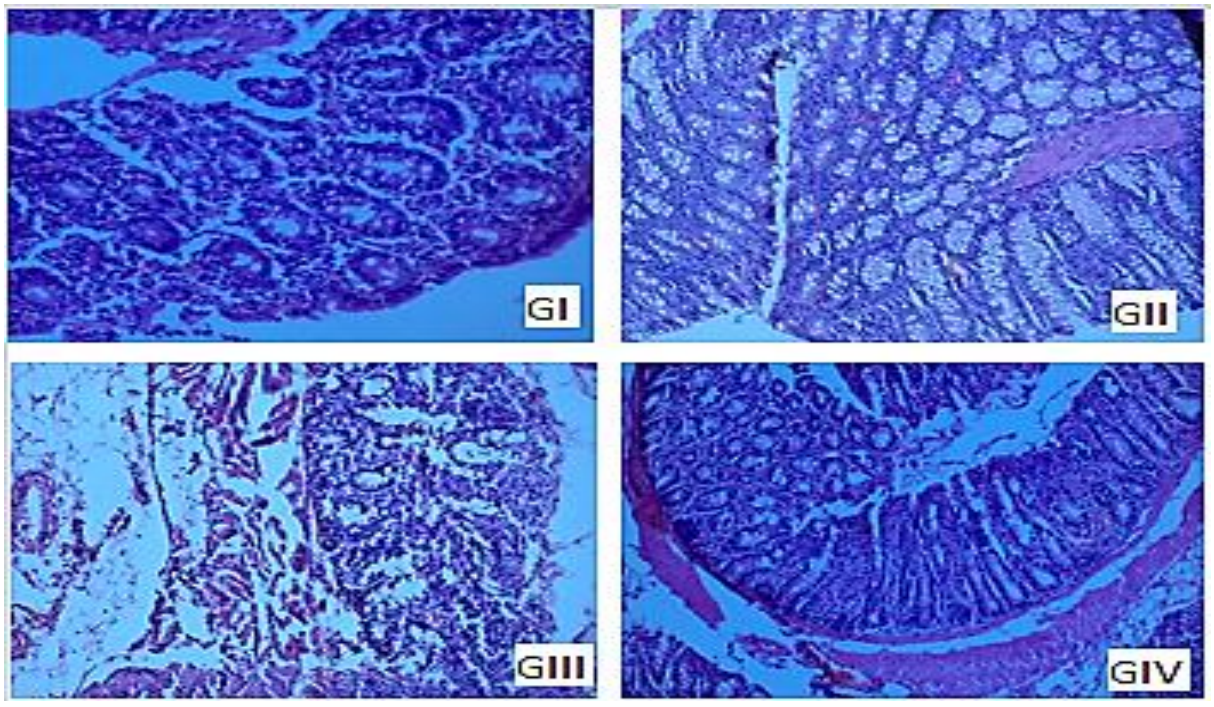

Fig. 3: Effects of FWEE on the prevention of inflammation and ulceration in the colon of different experimental groups.

3.6. Cyclooxygenase-2 expression studies

Cyclooxygenase-2 (COX-2) is considered a major facilitator of tissue inflammation because of its role in prostanoid production from the metabolism of arachidonic acid into prostaglandin H (PGH). In this study, Cox-2 levels were elevated in inflammation induced group 3 (Figure 4). Cox-2 occurrence was not observed in control and FWEE treated groups. Significant Cox-2 low was found in DSS+FWEE treated group. Since elevated COX-2 expression was found in most colorectal cancer tissue and is associated with worse survival among colorectal cancer patients, investigators have sought to evaluate the effects of non-steroidal anti-inflammatory drugs and selective COX-2 inhibitors on colorectal cancer prevention and treatment. Resulting is very interesting in this study and confirms the significant healing influence of FWEE.

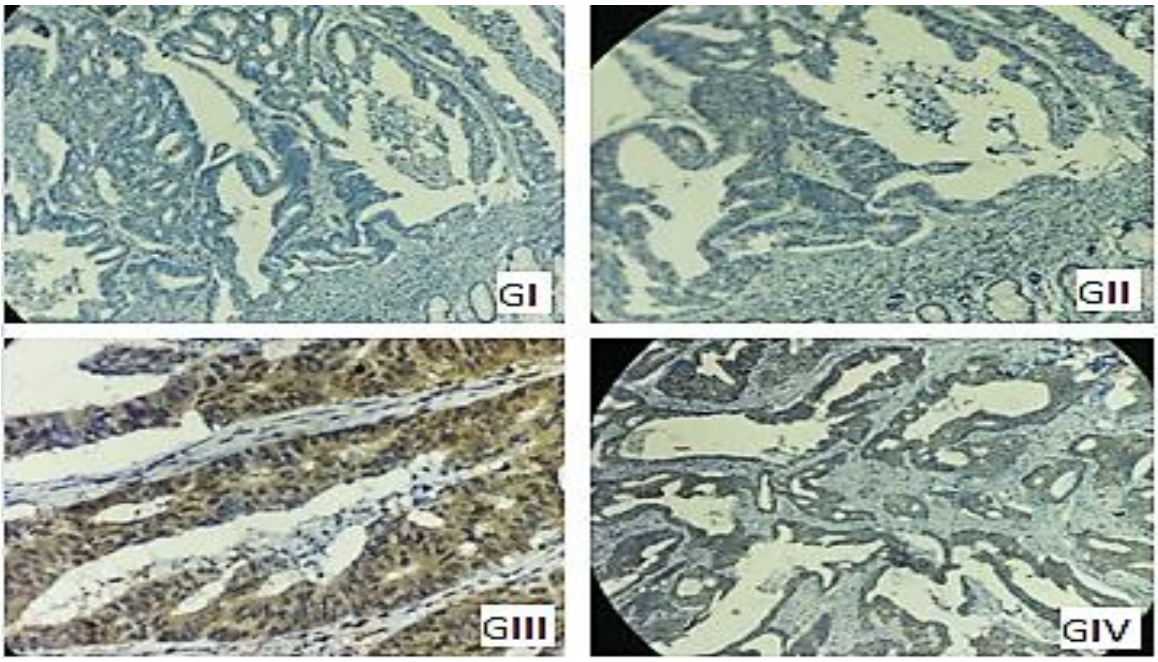

Fig. 4: FWEE suppresses the players involved in inflammation (Cox-2). The marker is higher in the DSS-treated group (G-III) and suppressed when the DSS-treated groups were fed FWEE (G-IV)

4. CONCLUSION

From these results, it can be concluded that the ethanolic extract of *F. wightiana* shows a significant anti-oxidant and anti-inflammatory activity which was demonstrated through DPPH, H_2O_2 and DSS model respectively. It can be concluded that the Ethanolic extract of leaves of *F.wightiana* have shown prominent anti-inflammatory property. Further studies are in progress towards the isolation of the active constituent responsible for this activity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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