

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF AERIAL PART EXTRACTS OF *PLANTAGO MAJOR* L.JYOTI VANDANA^{1*}, GUPTA A K², ALOK MUKERJEE³¹Department of Chemistry, SHUATS, Allahabad, Uttar Pradesh, India. ²Department of Forensic Science, SHUATS, Allahabad, Uttar Pradesh, India. ³Department of Pharmacognosy, United Institute of Pharmacy, Allahabad, Uttar Pradesh, India. Email: jyoti.mani@shiats.edu.in

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ABSTRACT

Objective: This study aims to perform phytochemical screening and investigate anti-inflammatory activity of extract from aerial part of the plant *Plantago major* L.**Methods:** Preliminary phytochemical screening of plant extract was carried out using standard qualitative procedures and anti-inflammatory activity was evaluated using carrageenan-induced paw edema model in male Wistar albino rats using plethysmometer. Diclofenac sodium (10 mg/kg, b.w.) was used as standard drug.**Results:** Preliminary phytochemical screening of the different extracts revealed that they were rich in secondary metabolite compounds such as carbohydrates, tannins, steroids, flavonoids, terpenoids, glycosides, fats and oils, alkaloids, phenolic compounds, and iridoid glycosides. The anti-inflammatory activity was evaluated using carrageenan-induced paw edema models in Wistar albino rats. The anti-inflammatory activity of methanolic extract (ME) was found to be dose dependent in carrageenan-induced paw edema model. The ME has shown significant ($p < 0.05$) inhibition of paw edema, 61.11% and 55.55% on 4th hr at the doses of 900 and 600 mg/kg, respectively.**Conclusion:** The results of the present study demonstrate that ME of the aerial parts of the plant possess significant ($p < 0.05$) anti-inflammatory potential.**Keywords:** Phytochemical screening, *Plantago major* L., Anti-inflammatory activity, Carrageenan, Diclofenac sodium.© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2017.v10i9.20031>

INTRODUCTION

In recent times, phytochemical and pharmacological investigations have acquired great information and knowledge for the use of medicinal plants by revealing the presence of active principles and actions on humans and animals. There is necessity to increase the efforts in screening and collecting plants for the development of new, environmentally friendly, and safe medicinal agents [1]. Inflammation is an important physiological reaction, which occurs in response to injury of cells and body tissues through different factors such as infections, chemicals, thermal and mechanical injuries by limiting damage, and promoting tissue repair [2]. When inflammatory process takes place, it protects our body from diseases by releasing cells and mediators that combat foreign substances and prevent infection. It is noted that excessive inflammation leads to rheumatoid arthritis, psoriasis, and other inflammatory diseases [3]. The five basic symptoms of inflammation are redness, swelling, heat, pain, and deranged function known since the ancient time. Inflammation is categorized as acute and chronic, where acute inflammation is the initial stage of inflammation that persists for short time, whereas when the inflammation is for longer period, it is termed as chronic inflammation. Depending on the intensity of inflammation, mediators generated in the affected area can reach the circulation and cause fever. Inflammation is a complex pathophysiological process, mediated by a variety of signaling molecules. These molecules produced by leukocytes, macrophages, and mast cell are responsible for the generation of inflammatory mediators such as nitric oxide, prostaglandin (PG) E₂, and tumor necrosis factor- α that bring about edema formation as a result of extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site [4]. The drugs used in the treatment of inflammation are non-steroidal anti-inflammatory drugs (NSAIDs) and SAIDs. These drugs have toxic effects such as ulceration and bleeding, renal damage,

hypertension, hyperglycemia, etc [5]. Therefore, continuous research is done to reduce such side effects. Whereas medicinal plants are known as alternate and important natural source of chemical substances with potential therapeutic effects as pain relievers and anti-inflammatory agents with less side effects.

Plantaginaceae is one of the most diverse family of plants [6]. The genus *Plantago* L. comprises 265 species with a worldwide distribution [7]. In Asia and Europe, *Plantago* species have been used in folk medicine for the treatment of various diseases such as wound healing, problems relating to digestive tracts, and asthma as a diuretic and anti-inflammatory drug [8,9]. *Plantago major* belongs to the genus *Plantago*, family Plantaginaceae and is commonly known as plantain. It is an old medicinal plant known for centuries [10]. It was discovered about 4000 years ago in Europe, but are also found in some parts of Northern and Central Asia and has widely distributed worldwide, particularly in temperate, but also tropical parts. It is a small perennial plant with height about 15 cm, but the size varies depending on the growth habitats [11]. In general, people call it as medicinal weed [12]. It is well-known traditional herbal plant to treat various diseases including skin diseases, infectious diseases, reduce pain and fever, reproduction, cancer, respiratory and digestive organs related problems [8]. In addition to the traditional uses of *P. major*, some researchers also prove its medicinal properties such as immune modulating property [13], antileukemia, antiviral, anticarcinogenic [14,15], antioxidant [16], anti-inflammatory, and analgesic activities [17]. These medicinal properties of *P. major* due to the presence of biologically active chemical compounds such as polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoid glycosides, terpenoids, alkaloids, and some organic acids [8,12,18]. Some studies reported that the leaves of *P. major* are used for its wound healing property, anti-inflammatory, antioxidant

activity, immunoenhancing property [8-13], and its seeds are used to treat various gastrointestinal disorders. The aim of the present study is to investigate the claimed folkloric information of plantain used for wound healing and other inflammatory problems using carrageenan-induced paw edema model for anti-inflammatory activity.

MATERIALS AND METHODS

Collection and identification of plant material

Aerial parts of *P. major* were collected from Forest Research Institute (FRI), Dehradun. It was identified and authenticated by Dr. (Mrs.) Veena Chandra, Head, Botany Division, FRI, Dehradun, Uttarakhand, India. A voucher specimen has been preserved in laboratory for the future reference with accession number 164439A/B/C.

Preparation of plant material

The collected plant material was washed thoroughly in water, air dried at room temperature under shade for 2 weeks, and pulverized in laboratory electric grinder. The powdered plant material was kept in air tight containers for further use.

Preparation of plant extracts

The shade-dried, powdered plant material was weighed and then successively extracted with solvents of increasing polarity starting with benzene, chloroform, ethanol, methanol by soxhlet extraction method, and separately, whole water extract was prepared. The obtained extracts were filtered separately and each extract was concentrated by distilling off the solvent and then evaporating it to dry by placing it on a porcelain dishes on electric water bath. The percentage yield of extracts was calculated and kept in air tight containers for pharmacognostic and pharmacological studies.

Animals

Male albino rats of Wistar strain weighing 150-200 g were procured from the Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Lucknow, Uttar Pradesh, India. The animals were kept for acclimatization under laboratory conditions for 1 week before the start of the study. Animals were housed 6 rats per polyacrylic cage with paddy husk as bedding at temperature of 25°C±4°C and relative humidity of 50%-60% and light/dark cycle of 12 hrs/day [19,20] were used throughout the period of anti-inflammatory activity study. The animal in a group was recognized by mark of picric acid on the fur of each animal. Animals had free access to pellet feed and water *ad-libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee, United Institute of Pharmacy, Allahabad, India, as per the provisions made by CPCSEA, Government of India (Regd. No. 1451/PO/a/11/CPCSEA). Animals were fasted overnight before and during experiment.

Methods

Preliminary phytochemical screening

The preliminary phytochemical screening of *P. major* aerial part extracts was carried out qualitatively for the presence of steroids, tannins, flavonoids, saponins, alkaloids, carbohydrates, glycosides, fats and oils, phenols, and iridoid glycosides. The preliminary phytochemical screening for bioactive compounds was carried out by the standard methods [21-23]. The phytochemical examinations were performed on the liquid and dried extracts.

Evaluation of anti-inflammatory activity of *P. major*

The methanolic extract (ME) is the most potent extract selected for anti-inflammatory activity. The dose of ME was selected for anti-inflammatory activity on the basis of previously published literature [24-26]. The maximum tolerated dose (MTD) of *P. major* ME was found 2 g/kg [27]; therefore, the LD50 is greater than 2g/kg. The one-tenth of the MTD of the extract was selected for evaluation of anti-inflammatory activity [28]. Therefore, 300, 600, and 900 mg/kg doses of *P. major* extract were selected. The anti-inflammatory activity of the extract was done against carrageenan-induced rat paw edema model [29].

Experimental design

The animals were divided into five groups consisting of 6 rats in each group. Each group received a particular treatment such as control, positive control, and three different doses of ME. Before any treatment, each rat was weighed properly and the doses of the test samples, positive control, and control were calculated accordingly. Either of control vehicle, standard drug (diclofenac sodium), or methanolic crude extract was given orally. Acute inflammation was provided by injection of 0.1 ml of 1% carrageenan into the subplantar surface of rat hind paw. Group I: served as control, rats received 1 ml normal saline and 0.1 ml of carrageenan. Group II: (Positive control) rats received diclofenac sodium (10 mg/kg b.w.p.o.) [30] and 0.1 ml of carrageenan, Group III: (Test group), rat received ME of *P. major* (300 mg/kg b.w. p.o.) and 0.1 ml of carrageenan. Group IV: (Test group), rat received ME of *P. major* (600 mg/kg b.w. p.o.) and 0.1 ml of carrageenan. Group V: (Test group), rats received ME of *P. major* (900 mg/kg b.w.p.o.) and 0.1ml of carrageenan. The paw volumes were measured plethysmometrically [31] up to a fixed mark by displacement at 1, 2, 3, and 4 hrs, after the administration of the standard and test drugs. The percentage inhibition of inflammation was calculated as inhibition of edema volume [32] as follows:

$$\text{Percentage inhibition of inflammation} = \frac{(1 - V_t)}{V_c} \times 100$$

Where V_t is the average paw edema volume of extract of treated group or positive control group.

V_c is the paw volume of the control that received only vehicle.

Statistical analysis

The data obtained from the experimental work are expressed as mean ± standard deviation. The statistical analysis of the anti-inflammatory activity of plantain was analyzed using one-way analysis of variance followed by Dunnett's *t*-test. Differences between the mean of treated animals and control group were considered statistically significant at $p < 0.05$ for *t*-test.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Phytochemicals are plant chemicals known to play an important role in biological activity of medicinal plants. There is an increasing interest in phytochemicals present in *P. major* due to their potential use in food supplements and pharmaceuticals. It has different phytochemicals in its leaves, seeds, stem, and roots, which really have restorative properties and can likewise be utilized as taxonomic markers [8]. The preliminary phytochemical screening was done to identify the secondary metabolites present in the different solvent extracts.

The results of qualitative phytochemical screening as given in the Table 1 shows the presence of phytochemicals such as carbohydrates, reducing sugar, alkaloids, flavonoids, tannins, steroids, terpenoids, glycosides, fats and oils, phenols, and iridoid glycosides whereas saponins and anthraquinones were absent in all the extracts. The result shows that carbohydrates, flavonoids, phenols, and iridoid glycosides were present in high amount as compared to other phytochemicals investigated. The maximum phytochemicals were present in ME as compared to other extracts. The polarity of extraction solvent may influence the number of bioactive compounds obtained from the plant extract. It was reported that methanol was found more desirable to extract the phytoconstituents. It was studied that methanol was 20% more effective than ethanol and 73% more effective than water [33]. The phytochemicals identified gave an idea about the chemicals present in this plant which may be responsible for the pharmacological activities and the reason for their use as a traditional medicine worldwide. The phytochemical evaluation of various phytoconstituents were graded as very high (+++), moderate (++), low (+), and nil (-) based on the intensity of the colored reaction product. The extraction yield calculated for all extracts showed that methanol extract recorded higher percentage of yield. All these five extracts gave different chemical

Table 1: Preliminary phytochemical screening of different solvent extracts of aerial part of *Plantago major*

| S.No. | Name of test | Benzene extract | Chloroform extract | Ethanol extract | Methanol extract | Water extract |
|-------|--------------------------|-----------------|--------------------|-----------------|------------------|---------------|
| 1 | Carbohydrates | | | | | |
| | Molisch's test | + | + | ++ | ++ | - |
| | Barfoed's test | - | + | + | ++ | - |
| 2 | Fehling's test | - | + | + | +++ | + |
| | Tannins | | | | | |
| | Ferric chloride test | - | - | + | ++ | - |
| 3 | Lead acetate test | - | - | + | + | - |
| | Steroids | | | | | |
| 4 | Liebermann-Burchard test | + | + | + | + | - |
| | Anthraquinones | | | | | |
| 5 | Borntrager's test | - | - | - | - | - |
| | Saponins | | | | | |
| 6 | Foam test | - | - | - | - | - |
| | Flavonoids | | | | | |
| 7 | Ferric chloride test | - | - | + | +++ | + |
| | Sodium hydroxide test | - | - | + | ++ | - |
| 8 | Terpenoids | | | | | |
| | Salkowski test | + | - | + | ++ | - |
| 9 | Alkaloids | | | | | |
| | Dragendorff's test | - | - | + | + | - |
| 10 | Mayer's test | - | - | + | + | - |
| | Glycosides | | | | | |
| | Keller-killiani's test | - | + | + | ++ | - |
| 11 | Salkowski test | - | - | + | + | + |
| | Borntrager's test | - | - | - | - | - |
| | Phenols | | | | | |
| 12 | Ferric-chloride test | - | - | ++ | +++ | + |
| | Lead acetate test | - | - | + | + | - |
| 13 | Fats and oils | | | | | |
| | Stain test | + | + | - | - | - |
| 14 | Iridoid glycosides | | | | | |
| | Trim and Hill's test | - | - | + | +++ | - |

-: Nil, +: Low, ++: Moderate, +++: Very high

composition due to different polarity of the extraction solvent. It may be due to high polarity of methanol solvent which can describe high variety of plant constituents than the other solvents.

In vivo anti-inflammatory activity

Different solvent extracts of *P. major* were evaluated for the presence of phytochemicals before the anti-inflammatory study. The result in Table 1 shows that among all the extracts, ME gave positive tests for most of the phytoconstituents during phytochemical screening. The extract was also selected because of its high extractive value. Therefore, the ME is the most potent extract selected for anti-inflammatory activity. Carrageenan-induced paw edema as an *in vivo* model of inflammation has been used to assess the anti-inflammatory activity of the test extract. The effect of extract on carrageenan-induced edema at different hours of study was compared to that of control for the evaluation of anti-inflammatory activity on the basis of percent inhibition of paw edema volume.

Carrageenan induced inflammation model is a useful acute experimental model for the estimation of anti-inflammatory effect. The development of edema in the paw of the rat after the injection of carrageenan is due to the release of histamine, serotonin, and Prostaglandin (PG) [34]. Carrageenan is phlogistic agent which induced edema develops by mediators in two phases, so inflammation produced by carrageenan has a biphasic effect. The first phase is due to release of mediators such as histamine and serotonin occurs within 0-2 hrs [35] and the second accelerating phase edema is attributed to bradykinins and PG release (>4th hr) [36]. The results of the present study showed that methanol extract of *P. major* significantly inhibited the formation of the rat paw edema in the early and late phases. Most of the anti-inflammatory medicines are effective in the last phase of edema formation [37]. As shown in Table 2, standard drug diclofenac sodium showed significant inhibition of edema at 4th hr (81.48%). Our results of anti-inflammatory activity as given in Table 2 confirmed that the ME significantly reduced

both phases of the carrageenan-induced edema. The significant anti-inflammatory activity has shown by methanol extract at higher concentration (at dose of 900 mg/kg body weight) which was comparable to standard. In this study, ME showed dose-dependent activity in both the phases of inflammation. The *P. major* inhibited the edema during acute phase of inflammation is possibly by inhibiting the mediators of inflammation.

The anti-inflammatory activity of *P. major* extract may be due to the presence of phytoconstituents given in Table 1. Some researchers reported that *P. major* contains biologically active chemical compounds such as polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoids glycosides, and terpenoids. Alkaloids and some organic acids have also been identified in this plant [8-18]. This study also observed that highest anti-inflammatory activity was found in the ME at a dose of 900 mg/kg. This result was similar to the findings of Beara *et al.* [18] and Hussan *et al.* [38]. It is reported that anti-inflammatory activity of *P. major* is contributed by flavonoids such as baicalein and hispidulin and iridoid glycosides such as aucubin [8]. Guillen *et al.* [17] studied an aqueous extract of *P. major* leaves at a dose of 1 g/kg for anti-inflammatory activity. The study concluded that aqueous extract of *P. major* has anti-inflammatory and analgesic activities. In the other study, the ME of *P. major* seeds was investigated for anti-inflammatory activity. The findings of the study suggested that *P. major* seeds' extract has anti-inflammatory activity [39]. Reina *et al.* [10] study the *in vitro* effects of *P. major* extract, baicalein and aucubin, on human neutrophil respiratory burst activity. They concluded that *P. major* extract, baicalein and aucubin, significantly inhibited the production of reactive oxygen species from the neutrophils. Numerous studies have reported that anti-inflammatory and analgesic activities of medicinal plants have been related the presence of tannins, flavonoids, alkaloids, phenolic compounds [40,41], and iridoid glycosides [42,43]. Therefore, the anti-inflammatory activity observed in *P. major* may be due to the activity or activities of one compound or a combination of a number of compounds

Table 2: Effect of ME of aerial part of *Plantago major* on carrageenan-induced paw edema in rats

| Group | Treatment | Dose (mg/kg b.w.p.o.) | Increase in paw volume (ml) | | | |
|-------|------------------|-----------------------|-----------------------------|---------------------|---------------------|---------------------|
| | | | 1 hr | 2 hrs | 3 hrs | 4 hrs |
| I | Control | 1 ml NS | 0.33±0.017 | 0.42±0.015 | 0.51±0.023 | 0.54±0.023 |
| II | Positive control | 10 | 0.15±0.011* (54.55) | 0.15±0.020* (64.29) | 0.13±0.01* (74.50) | 0.10±0.01* (81.48) |
| III | ME | 300 | 0.23±0.020* (30.30) | 0.24±0.028* (42.85) | 0.26±0.01* (49.01) | 0.27±0.02* (50.00) |
| IV | ME | 600 | 0.21±0.020* (36.36) | 0.22±0.030* (47.61) | 0.23±0.011* (54.90) | 0.24±0.015* (55.55) |
| V | ME | 900 | 0.19±0.005* (42.42) | 0.20±0.01* (52.38) | 0.21±0.005* (58.82) | 0.21±0.026* (61.11) |

All the results are expressed as mean±SD of six animals in each group; number in parenthesis indicates percentage inhibition in increase in paw volume. *p<0.05 when compared with control, statistically significant. ME: Methanolic extract, SD: Standard deviation, NS: Normal saline

belongs to the different classes of compounds present in aerial parts of *P. major* [44]. Further studies are in process for the isolation of active constituents responsible for anti-inflammatory activity and its possible mechanism.

CONCLUSION

The present study has shown that the *P. major* is a rich source of phytochemicals. The ME of the aerial part of plantain possessed a significant anti-inflammatory effect on paw edema induced by carrageenan. The carrageenan-induced inflammation model is a significant model for anti-inflammatory agents acting by the mediators of acute inflammation. The present study concluded that *P. major* is a rich source of phytoconstituents and effective in acute inflammatory disorders and is suggested for continual use as herbal anti-inflammatory medicine.

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