Analgesic, anti-inflammatory, antioxidant activity and phytochemical screening of *Dryopteris blanfordii* plant

Jan Sher, Gul Jan, Cai zhiquan, Muhammad Israr, Farzana Gul, Siraj Khan, Muddaser Shah and Shakir Ullah

Abstract

We investigated the scientific base for its traditional use in pain, inflammation, antioxidant and phytochemical potential of *Dryopteris blanfordii*. The powdered plant was extracted by the method of cold maceration using (methanol, ethanol and aqueous) as solvents. The acetic acid induced writhing test model was used for analgesic activity. Anti-inflammatory activity was evaluated by carrageenan-induced mice paw edema. For antioxidant activity we used 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power assay method respectively. In qualitative analysis, the phytochemical compounds such as carbohydrates, alkaloids, phenols, flavonoids, saponins, tannins and quinine were screened by using standard methods. In analgesic activity after the injection of ethanolic extract at a dose of 300mg/kg the writhing was reduced to (21±0.13) /5 minutes and showed 45% inhibition, compared with standard drug aspirin which reduced to (14±0.21) /5 minutes and having 40.43% inhibition. The anti-inflammatory activity at a dose of 300 mg/kg during drug administration after four hours showed significant different which compared with standard drug diclofenac sodium (10mg/kg) and 100, 200mg/kg. High dose at 300mg/kg reduced (1.04±0.03) after the 4th hours and standard drug diclofenac sodium reduced (0.08±0.04) after 4th hours. The maximum rate of inhibition of antioxidant was observed in the methanolic extract. In the qualitative analysis of the ethanol extract showed highest amount of phenols. We concluded that the *D. blanfordii* plant showed high potential of inflammation, antioxidant and biochemical compounds.

Keywords: *Dryopteris blanfordii*, antioxidant, phytochemicals, DPPH

1. Introduction

Pharmacology is the branch of biology which deals with the study of action of drug on living tissue, where drugs is defined as any man-made, natural molecule which relates a biochemical or physical effect on the cell, tissue, organ and organism. In drug discovery, medicinal herbs has been considered the important source of pharmaceuticals, employed the action of human diseases due to their excessive chemical variety and wide biological functionality (Nasrabad et al., 2012) [12]. Now a day, great cares have been rewarded to eco-friendly and bio-friendly plants, which can checked and remedy several human diseases (Onsare et al., 2013) [15]. In many cases, these man-made drugs cause side effects or adverse responses. As a result, more attention was given to the study then use of medicinal plants has remained taken place through the last two decades. The new isolation methods and pharmacological testing processes, new plant drugs originate their way into new drug. Such use of single unadulterated compounds with artificial medicines (Tress & Evans, 2003) [22]. Pteridophytes are the primitive land plants group on earth and established large group of vascular cryptograms. The position of the pteridophytes, between the lower cryptograms and higher vascular plants. Pteridophytes have a long ecological history on earth planet. They were known as far back as 380 million years ago. In our neighbor country India, Pteridophytes are mostly distributed in the Himalayan and coastal regions (Upreti et al., 2009) [23]. Pteridophytes also showed pharmaceutical ability and many of them are being used therapeutically (Kumar & Kaushik, 1999) [10]. The rural societies, ethnic groups and traditional throughout the world are using plant parts like rhizome, stem, fronds, pinnae and spores in various ways for the usage of several traditional since early time. Many researches are working on taxonomy, ecology and distribution of pteridophytes has been published from time to time but enough responsiveness has not been paid towards their pharmaceutical useful aspects (Dixit, 2001) [3]. In the present study efforts have been made to search medicinally important pteridophytes and properly recognized their useful feature.
2. Materials and methods

2.1 Plant Collection and Identification

*Dryopteris blanfordii* was collected from District Dir (Upper) in September 2017 and identified with the help of flora of Pakistan and compared with the already specimen present in the herbarium of Hazara University Mansehra.

2.2 Drying and powdering

The collected plant specimen was clean from dust and damage parts were removed and then shade dried for 25 days. The dried specimen was powdered with the help of electric grinder and then the powdered material was stored in air tight bottle and weighed in selected amounts (Kumaran, 2006) [11].

2.3 Preparation of extracts

The powdered was used for the preparation of extracts using methanol, ethanol and aqueous in continuous method respectively for 48 hrs. The extract was filtered through watchman No.1 filter paper. The solvent was recovered by rotary vacuum evaporator and the concentrated extracts were further evaporated to get dry extracts. The dried extract was stored in an airtight bottle (Ismail et al., 2012) [8].

2.4 Chemicals used

Methanol, ethanol, carrageenan, acetic acid and DPPH were used.

2.5 Animal used

Young and healthy Swiss albino mice of 25-30 gm weight at the age of one month were selected for the studies. The mice were procured from animal house of NIH (National Institute of Health) Islamabad. Mice were kept in hygienic condition in polypropylene cages and feed at standard supplement obtained from NIH. The room temperature were kept at 25-30 °C and acclimatized at 4 days.

3. Analgesic activity

For in vivo analgesic activity the acetic acid induced writhing method was used.

3.1 Acetic acid induced writhing method

Acetic acid induced writhing test was carried out for analgesic activity. The mice were divided into five groups. Mice of group 1st (control group) was injected 1ml normal saline (1% v/v). Then 1ml acetic acid was injected to all mice of group 2nd, 3rd, 4th and group 5th. Ten minutes after then injected acetic acid solution, the writhing were count for 5 minutes. After the writhing the group 2 mice were injected 1ml of aspirin solution. The mice of group 3rd, 4th and 5th were injected 1ml ethanolic extract solution at the dose of 100 mg/kg, 200 mg/kg and 300 mg/kg respectively. After the inhibition of writhes were determined (Podder et al., 2011) [18]. Finally the percentage (%) of analgesic activity was used by the following formula;

\[
\text{% inhibition} = \frac{\text{No. of writhings in tested drug}}{\text{No. of writhings in control}} \times 100
\]

3.2 Anti-inflammatory activity

Carrageenan induced paw edema test model was carried out for anti-inflammatory activity. Ethanolic extract of *D. blanfordii* was tested for anti-inflammatory activity against carrageenan paw edema in mice. Mice were divided into five groups. Mice of group 1st (control group) was injected normal saline (1% v/v). Then 1ml carrageenan was injected to group, 3rd, 4th and 5th. Diclofenac sodium (dissolved in 10 ml water) and then 1ml diclofenac sodium was injected to group 2nd. The mice of group 3rd, 4th and 5th were injected 1ml ethanolic extract solution at the dose of 100 mg/kg, 200 mg/kg and 300 mg/kg respectively. The reduction of paw edema of mice was compared with standard drug (Olajide et al., 2000) [14]. The percent inhibition was calculated by the following formula

\[
\text{% inhibition} = \frac{\text{Vc} - \text{Vt}}{\text{Vc}} \times 100
\]

Vc represent the edema value of control group, “Vt” represent the edema volume of treated groups.

4. Antioxidant activity

The free radical scavenging activity of different extracts of *D. blanfordii* were checked, using stable free radical, DPPH (2, 2-diphenyl-1-picrylhydrazyl) or Reducing power or Superoxide anion radical scavenging activity was determined spectrophotometrically. The optical density of the oxidation reaction were measured at 517 nm using spectrophotometer with solvent and DPPH as blank (Ayoolu et al., 2008) [8].

5. Phytochemical screening

The methanolic, ethanol and aqueous extract of *D. blanfordii* was screened by different chemical test for the identifying the basic chemical constituents present in the extract. The standard chemical tests for carbohydrates (Sharma et al., 2016) [20], alkaloid (Kavitha & Gunavathy, 2014) [9], phenols (Dahiri et al., 2006) [4], flavonoids (Zhao et al., 2007) [25], quinine (Savithramma et al., 2011) [19] and tannin (Zohra et al., 2012) [26] were performed by standard methods.

6. Statistical Analysis

The data were analyzed by one-way ANOVA (mean±SEM & mean±SD) followed by application of Duncan test (Pro 8 SRO v8.0724 (B724)), Northampton, MA, USA. A P value of <0.05 was considered as a statistically significant.

7. Results

7.1 Analgesic activity

The result of acetic acid induced writhing test was mentioned in a table 1. The mice of group 1(control group) showed writhing i.e (27.4±0.10) /5 minutes. Group 2nd mice significantly reduced writhing i.e 14±0.21 / 5 minutes and showed 40.43% inhibition. Among the group 3rd, 4th and 5th, the highest writhing counting was observed in Group 5th at the concentration of 300mg/kg (21±0.13 in /5 minutes and showed 55% inhibition reduction) followed by group 4th mice at a concentration of 200mg/Kg (28±0.32 /5 minutes) and showed 52.44% inhibition reduction (Table 1; Fig 1).

7.2 Anti-inflammatory activity

The initial paw edema volume was noted in all the mice of 5 groups i.e G1 (0.6±0.05), G2 (0.45±0.03), G3 (0.62±0.05), G4 (0.58±0.05), G5 (0.56±0.05) in mm³. After measuring the initial paw edema volume,1ml carrageenan was injected to all the mice of group 2 to 5. After one hour the paw edema volume were recorded i.e G1(1.12±0.03), G2 (1.96±0.03), G3 (1.84±0.05), G4 (1.94±0.03) and G5 (1.76±0.04). After the administration of carrageenan, the mice were injected standard drug (Group 2nd) and ethanolic extract at selected dose (G3 to G5). The results were noted at the interval of 1 hour. The group 2nd result was recorded 1.48±0.03, 1.11±0.03,
1.01±0.03 and 0.68±0.04 (mm³) after 1st, 2nd, 3rd and 4th hours respectively. The mice of group 3rd 1ml ethanolic extract was injected at a dose of 100mg/kg and the paw edema volume was recorded 1.53±0.05, 1.33±0.03, 1.24±0.05 and 1.63±0.05 (mm³) after 1st, 2nd, 3rd and 4th hours respectively. The mice of group 4th 1ml ethanolic extract was injected at a dose of 200mg/kg and the paw edema volume was recorded 1.69±0.03, 1.41±0.03, 1.37±0.03 and 1.22±0.03 (mm³) after 1st, 2nd, 3rd and 4th hours respectively. The mice of group 5th was injected 1ml ethanolic extract at a concentration of 300mg/kg and the paw volume was noted 1.65±0.03, 1.32±0.03, 1.2±0.03 and 1.04±0.03 (mm³) after 1st, 2nd, 3rd and 4th hours respectively. The group 5th mice at the concentration of 300mg/kg was observed a significant result at the 4th hours (1.04±0.03) followed by G3 and G4 (1.16±0.03) (Table 2; Fig 2).

7.3 Antioxidant activity
(DPPH radical scavenging activity)
Antioxidant activity of Dryopteris blanfordii was carried out by using methanolic, ethanolic and aqueous extracts. Extracts were subjected for the evaluation of antioxidant activity by using in vitro model systems. DPPH radical scavenging activity was observed in all the extracts.

7.3.1 % inhibition antioxidant activity of the Dryopteris blanfordii at concentration 1, 1.5 and 2mg/ml
The antioxidant activity of Dryopteris blanfordii % inhibition was carried out at concentration of 1 mg/ml. The high % inhibition of Dryopteris blanfordii was observed in the methanolic extract 55.6% (0.31±0.03) followed by the ethanolic extract 48.7% (0.52±0.05) and aqueous extract 30.5% (0.64±0.004). The % inhibition at 1.5mg/ml concentration the high % inhibition of Dryopteris blanfordii was observed in the methanolic extract 66.08% (0.58±0.11) followed by the ethanolic extract 54% (0.32±0.001) and aqueous extract 35.7% (0.63±0.01). The % inhibition at 2 mg/ml concentration, the high % inhibition was observed in methanolic extract 67.5% (0.34±0.05) followed by ethanolic extract 45% (0.51±0.06) and aqueous extract 25.9% (0.53±0.001) (Table 3; Fig 3).

8. Qualitative detection of bioactive compounds of Dryopteris blanfordii
Phytochemical detection of Dryopteris blanfordii in methanolic extracts showed the presence of carbohydrates, alkaloid, flavonoid, phenol, saponin and tannin, but quinine was found absent. Ethanolic extract showed the presence of carbohydrates, alkaloid, flavonoid, tannin phenol, saponin and quinine. In aqueous extract, the detection of phytochemical showed the presence of carbohydrates, alkaloid, flavonoid, phenol, quinine and tannin, but saponine was found absent (Table 4; Fig 4).

Table 1: Analgesic activity of ethanolic extract of Dryopteris blanfordii plant

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Number of writhing in 5 minutes (mean±SEM)</th>
<th>Percent inhibition of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>10ml/kg</td>
<td>27.4±0.10</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>G2</td>
<td>150mg/kg</td>
<td>Aspirin 14±0.21</td>
<td>40.43</td>
</tr>
<tr>
<td>G3</td>
<td>100mg/kg</td>
<td>30** ±0.10</td>
<td>60.74</td>
</tr>
<tr>
<td>G4</td>
<td>200mg/kg</td>
<td>28±0±0.32</td>
<td>52.44</td>
</tr>
<tr>
<td>G5</td>
<td>300mg/kg</td>
<td>21** ±0.13</td>
<td>45</td>
</tr>
</tbody>
</table>

*G’ stand for groups’ (Value are expressed in mean ±SEM) P* < 0.05 used as significant

Fig 1: Percentage (%) inhibition of writhing

Table 2: Anti-inflammatory activity in ethanolic extract of Dryopteris blanfordii plant.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Before carrageenin injection 1 hr. after carrageenin injection</th>
<th>Paw edema (mm³) after drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mean±SEM)</td>
<td>1 hr (mean±SEM)</td>
</tr>
<tr>
<td>G1</td>
<td>10mg/kg</td>
<td>0.65±0.05</td>
<td>1.13±0.05</td>
</tr>
<tr>
<td>G2 Diclofenac sodium</td>
<td>10mg/kg</td>
<td>0.45±0.03</td>
<td>1.96±0.03</td>
</tr>
<tr>
<td>G3</td>
<td>100mg/kg</td>
<td>0.62±0.05</td>
<td>1.84±0.05</td>
</tr>
<tr>
<td>G4</td>
<td>200mg/kg</td>
<td>0.58±0.05</td>
<td>1.94±0.03</td>
</tr>
<tr>
<td>G5</td>
<td>300mg/kg</td>
<td>0.56±0.05</td>
<td>1.76±0.04</td>
</tr>
</tbody>
</table>

G’ stand for groups P*<0.05 is significant which compared with standard drug.
Fig 2: Percentage inhibition of anti-inflammatory activity

Table 3: Antioxidant activity of Dryopteris blanfordii Plant

<table>
<thead>
<tr>
<th>Plant part used</th>
<th>Concentration</th>
<th>Extraction</th>
<th>Mean±SD</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole part</td>
<td>1 mg/ml</td>
<td>Methanol</td>
<td>0.31**±0.03</td>
<td>55.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
<td>0.542*±0.05</td>
<td>48.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>0.64**±0.004</td>
<td>30.58</td>
</tr>
<tr>
<td>Whole part</td>
<td>1.5 mg/ml</td>
<td>Methanol</td>
<td>0.58*±0.11</td>
<td>66.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
<td>0.52**±0.001</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>0.63**±0.01</td>
<td>35.7</td>
</tr>
<tr>
<td>Whole part</td>
<td>2 mg/ml</td>
<td>Methanol</td>
<td>0.34*±0.05</td>
<td>67.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
<td>0.51*±0.06</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>0.53**±0.001</td>
<td>25.9</td>
</tr>
</tbody>
</table>

Value are expressed in mean ±SD) P* < 0.05 used as significant

Fig 3: Percentage (%) inhibition of Antioxidant activity

Table 4: Qualitative detection of bioactive compounds in Dryopteris blanfordii Plant

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Phytochemical tests</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Phenol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Quinins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) show presence and (-) show absence of phytochemical

Discussion

In the present study the analgesic, anti-inflammatory, antioxidant activity and phytochemicals investigation of Dryopteris blanfordii was carried out. The ethanolic extract of Dryopteris blanfordii showed significant results in analgesic and anti-inflammatory activities. In analgesic activity at a dose of 300mg/kg showed significant effect (21±0.13) writhing and 45% inhibition as compared to standard drug aspirin showed (14±0.21) writhing with 40.43% inhibition. The analgesic activity showed high potential due to the presence of alkaloids, flavonoids and phenolic contents.
(Sultana et al., 2014) [21]. Alkaloids have been reported to possess analgesic, antispasmodic and bactericidal, antimalarial activities (Okwu and Okwu, 2004 [13]; Oomah, 2003) [6]. It was concluded that plant extract have high flavonoids which showed high analgesic effect and without flavonoids contents no analgesic effect (Oweyele et al., 2005). Anti-inflammatory activity at a dose of 300 mg/kg after four hour showed significant result followed by the dose of 100mg/kg, 200mg/kg and standard drug diclofenac sodium (10mg/kg). High dose of extract 300mg/kg reduce inflammation (1.04±0.03) after 4th hours followed by group 3rd at the concentration of 200mg/kg at 4th hours (1.22±0.03). Several reports are available on flavonoid groups which exhibited high potential biological activities such as analgesic, anti-inflammatory, antimicrobial, anti-angioic, anticancer and anti-allergic reactions (Anayasor et al., 2010 [11]; Igbinosa et al., 2009) [7]. Phytochemical detection in methanolic extracts showed positive result except quinine. In ethanolic and aqueous extract all test were positive but only carbohydrates and saponine do not showed positive result in aqueous extract. (Gracelin et al., 2013) [6] used methanolic extract of five species of ferns in family Pteridaceae. Among these selected ferns showed carbohydrates, alkaloids, flavonoids and phenol, saponins, glycosides and tannins. The antioxidant activity of D.blanfordii in 1mg/ml concentration showed different result. High percent inhibition showed in methanolic extract (55.6%) followed by the ethanolic extract (48.7%) and aqueous extracts (30.58%), while the percentage inhibition in 1.5mg/ml concentration mentioned in a table. The high percent inhibition was observed in methanolic extract (66.08%) followed by the ethanolic extract (45%) and aqueous (35.7%). The percent inhibition in 2mg/ml concentration were observed in methanolic extract (67.5%) followed by the ethanolic (45%) and aqueous (25.9%). Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers (Barile et al., 2007; Ayoola et al., 2008) [2, 3]. Flavonoids are known to possess antioxidant anticanccer, antiviral and anti-inflammatory properties (Valizadeh et al., 2015). Phenolic compounds are well known to possess biological activities such as antioxidant, anti-diabetic, hepatoprotective, anti-inflammatory, antimicrobial and anticanccer (Gracelin et al., 2013) [6].

Conclusion

In conclusion, the ethanolic extract of Dryopteris blanfordii was proved a natural safe remedy for the treatment of analgesia and inflammation. Our current findings demonstrated scientific rationale for the folk use of the plant as analgesic, anti-inflammatory and antioxidant potential. Interestingly the D.blanfordii exhibited both peripheral as well as central analgesic effect which might have been attributed to the presence of such active principles, due to which it has proven folk use in various nervous disorders. Nevertheless, the isolation of pure secondary metabolites from the plant will help us further in understanding the mechanism of these activities and identification of lead compounds of clinical utility.

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References


