Effect of *Plumbago zeylanica* on Analgesia and Arthritis
Subasini Uthirapathy1*

**Abstract**

**Background:** Rheumatoid arthritis is a chronic inflammatory joint condition that causes oxidative damage and inflammation. The current study evaluates the safety and effectiveness of an 85 percent methanolic extract of *Plumbago zeylanica* (PZ). **Methods:** The acute toxicity study is used to calculate the LD50 value in the safety profile. The hot plate and tail immersion procedures are both used to examine the analgesic effect in the efficacy profile. Paw edema caused by carrageenan was investigated for its ability to reduce inflammation and complete Freund’s adjuvant produced arthritis was tested for anti-arthritic activity. **Results:** Various phytochemical analyses, including qualitative and quantitative analyses of 85 % methanolic extract and raw materials. The extract exhibits analgesic effects by increasing the reaction time in both the hot plate and tail immersion procedures. In Freund’s adjuvant-induced paw edema, the anti-inflammatory activity of methanolic extract is 30.42 % and 16.90 %, respectively. **Conclusion:** The PZ extract raises the level of antioxidants and lowers the oxidative stress caused by arthritis caused by complete Freund’s adjuvant. PZ has a lot of secondary metabolites that can help with pain, inflammation, and free radicals.

**Keywords:** Phytoconstituents, Tail immersion method, Carrageenan, Hot plate method, Carrageenan, Complete Freund’s adjuvant

**Introduction**

Rheumatoid arthritis, 1.3 million Americans suffer from rheumatoid arthritis (RA). Synovial cell proliferation and inflammatory cell invasion of the joints are two features of the chronic inflammatory joint disease (Uthirapathy, S. 2023). In spite of the extensive study that has been done, the etiopathogenesis of RA is still unknown. Inflammation is the primary sign that someone has rheumatoid arthritis. Inflammatory responses are extremely significant to human health due to the fact that many prevalent debilitating illnesses, such as rheumatoid arthritis, arthralgia spondylolysis, and ankylosis, etc., are biological manifestations of a weakened or exaggerated inflammatory response. Inflammatory reactions are responsible for the production of prostaglandins, which are chemical messengers that play a role in the healing process. This is the case because inflammatory responses are involved in the pathogenesis of many common debilitating diseases. The most fundamental response that the body has to microbial infection, frustration, and other types of tissue harm is inflammation. Inflammation, warmth, swelling, an accumulation of leucocytes, as well as discomfort, are essential features of the condition. In addition, Kamanli, et al., (2004) have presented an explanation that accumulating clinical data provides persuasive evidence for the participation of radicals in RA. Diseases that are caused by

**Significance |** The inhibition activity of PZ on carrageenan-induced arthritis, humoral and cellular immunity, and pain and inflammation.

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inflammation and oxidative stress, such as rheumatoid arthritis, have prompted the pharmaceutical industry to search for medications that have anti-inflammatory properties in addition to analgesic and antioxidant capabilities. A wide range of herbs is being investigated for each of these activities (Vetrivelan, et al., 2013).

Plumbago zeylanica (PZ) is widely referred to in Tamil as ‘chittiramulam or vellai’ and is typically found in the southern regions of India. It belongs to the family Plumbaginaceae. This plant’s various components were susceptible to a variety of illnesses. It is widely employed as an anti-inflammatory, anti-allergic, antioxidant, and anti-fertility agent (Quality standards of Indian medicinal plants, (2004); Tamilarasi et al., 2011; Sharma et al., 2009; Firestein, (2003). The injection of carrageenan subcutaneously is intended to stimulate pain response and cause skin redness. This reaction happens owing to pro-inflammatory mediators like bradykinin, histamine, IL4, IL10 and TNF. These mediators migrate effortlessly to areas of inflammation, as demonstrated by the current study. In an animal model of paw edema, treatment of carrageenan elicited a spectacular inflammatory response (David and Michael, 2001). Inflammation is a condition characterized by complicated mediators and tissue destruction (Kantha Deivi, et al., 2010). These results indicate, however, that the methanolic extract of PZ possesses analgesic and anti-inflammatory effects and can minimize inflammatory inflammation and tissue damage. In the current study, rats were used to investigate the antinociceptive and anti-inflammatory effects of the methanolic extract of PZ herbs via central and peripheral pathways. These effects were evaluated using antinociceptive and anti-inflammatory measures.

Materials and Methods

Plant material

The Madurai district of Tamilnadu, India, was the location where the collection of roots of Plumbago zeylanica (L) (PZ) took place. The plant was identified and verified as genuine by the Division of Pharmacognosy at SASTRA University in Thanjavur, Tamil Nadu, India.

Extraction

Drying the plant material in the shade. Seven days were spent soaking one kilogram of crushed plant root in hexane, chloroform, ethyl acetate, and 85% methanol. The extract was refined and concentrated by distillation. At 50ºC and at low pressure, any residues of solvent were evaporated. The following is the yield from the extract: The percentages are as follows: 2.45% in hexane, 2.96 in chloroform, 1.78% in ethyl acetate, and 6.78 % in 85 % methanol.

Qualitative and Quantitative analysis of PZ

Alkaloids, flavonoids, glycosides, phenols, resins, saponins, tannins, phytotheroster, carbohydrates, and amino acids were all confirmed in the extracts using standard procedures (6). The raw herb and an 85% methanolic extract of PZ were tested for their various phytoconstituents using a UV spectrophotometer. Vitamins C (Chang, et al., 2008) and Vitamin E (Sarojini, Y, Nittala, S.S., 1999) were among them, as were phenol and tannin (Okwu, D.E., 2005), carbohydrates (Dubois, et al., 1954).

Acute toxicity study

There appear to be eight male and eight female mice in each of the ten groups. A dose of 100, 200, 300, 500, 700, 900, 1100, 2000, 3000, and 4000 units of extract were given to each group of animals, as shown in Table 3. The animals were observed for 24 hours, and deaths were recorded. Follow the steps below to find the LD50 (Uthirapathy, Subasini and Ahamad, Javed (2022).

Animals used in experiments

In this experiment, 96 adult Wistar albino rats weighing between 180 and 210 g were employed. They came from SASTRA University’s Central Animal House, which is part of the Centre for Advanced Research in the Indian System of Medicine (CARISM). The rats were fed a standard lab diet and provided with purified water prior to the experiment. The temperature (22 ± 10ºC) and lighting (12 hours of light and 12 hours of darkness) in the animal lab were controlled automatically. This study used animals, but only after getting permission from an animal ethics committee (Clearance No. 12/SASTRA/IAEC/RPP).

Methodological Protocol for the Experiment Involving a Hot Plate

Rats were placed into groups for the Hotplate test (Shabi, et al., 2014). Each group has six animals. Group 1 received 5% Tween 80 in water, Group 2 received 200 mg/kg paracetamol, Group 3 received 150 mg/kg PZ extract, Group 4 received 250 mg/kg extract, and Group 5 received 350 mg/kg extract. In this experiment, each animal was placed its own hot plate heated to 55± 0.3ºC. The nociceptive threshold was determined by timing the duration until the animal reacted with a jump or hind paws licking in order to escape the heat. After administering the medication, the nociceptive threshold was tested regularly for 4 hours, at 60-minute intervals.

Tail immersion experiment protocol

Tail immersion was done in groups of rats (Mohammed, et al., 2022) as follows. Six animals per group. Group 1 received 5% Tween 80 in water, Group 2 received 200 mg/kg paracetamol, and Group 3 received 150 mg/kg extract. Group 4 received 250 mg/kg b.wt. extract and Group 5 received 350 mg/kg. b.wt of extract. Rats were tested for antinociception using the Analgesiometer tail-flick test. PZ and the vehicle were used to elicit reactions every 15 mints till 60 min after treatment.
Carrageenan-induced inflammation test

Carrageenan-induced anti-inflammatory properties of 85% methanolic PZ extract were examined (Uthirapathy, Subasini., 2021; Subasini, et al., 2007). The animals were grouped as follows. Group 1 received 5% Tween 80 in water, Group 2 received 10 mg/kg b.wt. indomethacin, Group 3 received 150 mg/kg b.wt of extract, Group 4 received 250 mg/kg b.wt extract and Group 5 received 350 mg/kg b.wt of extract. The test sample was administered 1 hour before an intradermal injection of carrageenan (0.1 ml of 1% solution in 0.9% saline) into the right hind paw plantar region. The other paw received 0.1 ml saline. Volume displacement was used to quantify paw volume before and after injection for 4 hours. Inflammation was measured by left-right paw volume differences. For average paw volume increase, they were compared to the saline and indomethacin groups.

Freund’s adjuvant caused arthritis experimental procedure

The animals were split up into three separate groups. There are six animals in each group. Group 1 animals were given 5% Tween 80 in water, Group 2 animals were given Complete Freund’s adjuvant (CFA), and Group 3 animals were given 50 mg/kg b.wt extracts in addition to CFA. 0.05 ml of CFA was injected under the skin (subcutaneously) of each rat's right foot pad (Uthirapathy, S. and Tahir, T. F., 2021). The animals were given an extract and a standard drug for 45 days. Under the influence of ether, all of the animals were sacrificed via cervical dislocation on day 46. After removal, the organs were washed in saline water. From a 10% tissue homogenate in Tris buffer, several biochemical parameters including Thio barbituric acid Reactive Substances (TBARS) (Okhawa, W.O, Yagi, K., 1979), Reduced Glutathione (GSH) (Elliott, G.L., 1959), Glutahtione Peroxidase (GPx) (Rotruck, et al., 1973), and Catalase (Sinha, A.K., 1972) were determined.

Statistical Analysis

The values are presented as Mean ± S.D. We used the student’s t test to see if there was a significant difference (p<0.05) in the size of the paws in Freund's adjuvant-induced arthritis.

Results

The results of hexane extract of PZ are not a good source of secondary metabolites, but a chloroform extract has flavonoids and phytosterol and an ethyl acetate extract has phenolic compounds and phytosterol. In a similar manner, the extract made up of 85% methanol seems to be a rich source of phenolic compounds, phytosterol, tannins, carbohydrates, and amino acids. The 85% of methanolic extract of PZ was selected for additional research since it contained a significant quantity of the majority of the secondary metabolites. Phytoconstituents such as phenol, tannin, carbohydrate, Vitamin C, and Vitamin E were measured in both the raw materials and the 85% methanolic extract. The raw materials have a larger carbohydrate concentration, but the 85% methanol fraction has a higher concentration of Vitamin C, followed by Vitamin E and tannins (data not shown). The acute toxicity analysis of 85% methanolic extract PZ shows no deadly impact on mice up to a dose of 4000 mg/kg body wt. The extract's ED50 value was beginning to decline between 200 and 400 mg/kg body weight. In the acute toxicity testing for the methanolic extract of the PZ herb extract, no further evidence of toxicity was seen (Data not shown).

According to the findings shown in Table 1, the quantity of activity produced by the PZ extract at the lower dosage was the lowest in the first hour. When the dose is increased to 250 mg/kg of body weight, a substantial difference and peak result are found on the second hour itself. When the dose is increased to 350 mg/kg of body weight, the maximum effect is observed on both the first and second hours. In addition to this, a high dose of extract activity, at 350 mg/kg b.wt. was observed. When using the tail immersion method, the reaction time starts to become noticeably slower (p < 0.05, Table 2). After waiting thirty minutes after administration the drug, the animals having the strongest effect. There was not a detectable increase in response time that was dose dependent.

Animals treated with 150 mg/kg b.wt. extract show no significant improvement in carrageenan-induced paw redness. At 250 mg/kg b.wt. of extract, a substantial difference has been seen and the response is considered to be amplified. Additionally, PZ only displays 30.42% activity at 250 mg/kg body weight of extract, whereas indomethacin exhibits 50.20% activity (Table 3).

It is known that animals given PZ for 45 days had smaller paw volumes. The significant difference was found on the 15th day of therapy, and the major difference was seen on the 45th day. On the 45th day, PZ therapy demonstrates 16.90% activity, but indomethacin treatment exhibits 29.92%. Table 4 displays the results.

The results in Table 5 show that the level of TBARS is beginning to rise in the livers of animals with arthritis. It is known that giving PZ to animals lowers the level of TBARS by a large amount (p<0.05, Table 5). In the liver of disease group animals, antioxidants such catalase, reduced glutathione, and glutathione peroxidase continue to be decreased. On the other hand, all of these antioxidants are found to be higher in animals that are treated with PZ. Low amounts of catalase and glutathione were detected in kidney tissue in order to protect other organs from CFA-induced damage. The PZ extract treatment significantly increases catalase levels (p<0.05, Table 5), but glutathione levels are still found to be lower. This power results from the rapid release of glutathione into the circulation to prevent the entire body from the injury done by CFA.
Table 1. Hot plate evaluation of Plumbago analgesic efficacy in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Reaction time in hr.</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>-</td>
<td></td>
<td>3.3 ± 1.41</td>
<td>4.0 ± 0.50</td>
<td>4.3 ± 0.57</td>
<td>4.4 ± 0.58</td>
<td>2.3 ± 0.50</td>
</tr>
<tr>
<td>Group II</td>
<td>Paracetamol 200 mg/kg</td>
<td>4.2 ± 1.73</td>
<td>5.9 ± 1.03</td>
<td>7.5 ± 0.57</td>
<td>6.5 ± 1.84</td>
<td>5.3 ± 1.26</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>MLE 150 mg/kg</td>
<td>4.0 ± 1.41</td>
<td>5.5 ± 1.59</td>
<td>5.7 ± 1.59</td>
<td>5.6 ± 1.71</td>
<td>4.8 ± 1.25</td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>MLE 250 mg/kg</td>
<td>4.5 ± 1.73</td>
<td>5.4 ± 0.75</td>
<td>6.5 ± 1.00*</td>
<td>5.6 ± 1.71</td>
<td>5.6 ± 0.64</td>
<td></td>
</tr>
<tr>
<td>Group V</td>
<td>MLE 350 mg/kg</td>
<td>3.8 ± 1.73</td>
<td>6.2 ± 0.95*</td>
<td>7.8 ± 1.70**</td>
<td>3.9 ± 0.90</td>
<td>5.6 ± 0.58</td>
<td></td>
</tr>
</tbody>
</table>

Note: * P < 0.01, **P < 0.001

Table 2. Evaluation of Plumbago's analgesic efficacy on rats by the Tail Immersion.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Reaction time in min.</th>
<th>0 min.</th>
<th>15 min.</th>
<th>30 min.</th>
<th>45 min.</th>
<th>60 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>-</td>
<td></td>
<td>4.3 ± 0.50</td>
<td>4.0 ± 0.80</td>
<td>3.8 ± 0.50</td>
<td>5.3 ± 0.96</td>
<td>4.0 ± 0.80</td>
</tr>
<tr>
<td>Group II</td>
<td>Standard 200 mg/kg</td>
<td>3.8 ± 0.96</td>
<td>4.3 ± 1.50</td>
<td>4.4 ± 1.10</td>
<td>5.3 ± 1.30</td>
<td>5.1 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>MLE 150 mg/kg</td>
<td>2.8 ± 0.50</td>
<td>4.3 ± 0.50</td>
<td>3.5 ± 1.00</td>
<td>4.3 ± 0.96</td>
<td>4.9 ± 0.90</td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>MLE 250 mg/kg</td>
<td>2.6 ± 0.50</td>
<td>3.5 ± 1.00</td>
<td>4.8 ± 1.30*</td>
<td>4.1 ± 0.90</td>
<td>4.0 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Group V</td>
<td>MLE 350 mg/kg</td>
<td>3.1 ± 0.30</td>
<td>4.3 ± 0.50*</td>
<td>4.0 ± 0.80</td>
<td>4.4 ± 1.10</td>
<td>4.1 ± 0.90</td>
<td></td>
</tr>
</tbody>
</table>

Note: * P < 0.01, **P < 0.001

Table 3. Plumbago's effect on carrageenan-induced rat paw edema.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg b.wt</th>
<th>Increase in Paw edema (Mean ± SD) in mm</th>
<th>% Inhibition of Paw edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>5 % Tween 80</td>
<td>2.00 ± 0.14</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>0.99 ± 0.10</td>
<td>50.20</td>
</tr>
<tr>
<td>Group III</td>
<td>150</td>
<td>1.58 ± 0.25</td>
<td>20.80</td>
</tr>
<tr>
<td>Group IV</td>
<td>250</td>
<td>1.39 ± 0.12*</td>
<td>30.42</td>
</tr>
<tr>
<td>Group V</td>
<td>350</td>
<td>1.75 ± 0.15</td>
<td>12.96</td>
</tr>
</tbody>
</table>

Note: * P < 0.01, **P < 0.001
### Table 4. Effect of Plumbago on Freund’s complete adjuvant induced inflammation

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>Indomethacin</th>
<th>Plumbago zeylanica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. V</td>
<td>P. V</td>
<td>P. I</td>
</tr>
<tr>
<td>1</td>
<td>4.27±0.08</td>
<td>4.32±0.11</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>6.3±0.43</td>
<td>6.2±0.33</td>
<td>1.59</td>
</tr>
<tr>
<td>15</td>
<td>6.68±0.51</td>
<td>6.38±0.41</td>
<td>4.49</td>
</tr>
<tr>
<td>22</td>
<td>6.7±0.87</td>
<td>6.08±0.25</td>
<td>9.25</td>
</tr>
<tr>
<td>29</td>
<td>6.7±0.46</td>
<td>5.85±0.19*</td>
<td>12.69</td>
</tr>
<tr>
<td>36</td>
<td>6.63±0.32</td>
<td>4.78±0.19*</td>
<td>27.90</td>
</tr>
<tr>
<td>43</td>
<td>6.45±0.39</td>
<td>4.52±0.11*</td>
<td><strong>29.92</strong></td>
</tr>
</tbody>
</table>

Note: P.V – Paw volume in mm; P.I – Percentage Inhibition

### Table 5. Plumbago’s impact on oxidative stress caused by Freund’s complete adjuvant

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Group 1 Normal</th>
<th>Group 2 Control</th>
<th>Group 3 Standard</th>
<th>Group 4 Plumbago</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS</td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td></td>
<td>0.47±0.05</td>
<td>0.29±0.04</td>
<td>0.82±0.03*</td>
<td>0.34±0.03</td>
</tr>
<tr>
<td></td>
<td>0.47±0.09*</td>
<td>0.40±0.10</td>
<td>0.61±0.10</td>
<td>0.71±0.36</td>
</tr>
<tr>
<td>Catalase</td>
<td>17.60±0.59</td>
<td>15.24±1.81</td>
<td>11.23±0.72</td>
<td>12.49±0.56*</td>
</tr>
<tr>
<td></td>
<td>16.43±1.24*</td>
<td>15.40±1.41*</td>
<td>15.14±0.22*</td>
<td>12.07±0.16</td>
</tr>
<tr>
<td>GSH</td>
<td>25.85±0.39</td>
<td>26.67±2.65</td>
<td>19.74±2.45</td>
<td>25.72±3.99</td>
</tr>
<tr>
<td></td>
<td>25.23±2.93</td>
<td>27.56±1.38</td>
<td>21.86±0.59</td>
<td>22.96±4.29</td>
</tr>
<tr>
<td>GPx</td>
<td>0.186±0.0007</td>
<td>0.112±0.0001</td>
<td>0.107±0.0001</td>
<td>0.112±0.0001</td>
</tr>
<tr>
<td></td>
<td>0.141±0.0001*</td>
<td>0.113±0.0015</td>
<td>0.132±0.0002*</td>
<td>0.112±0.0001</td>
</tr>
</tbody>
</table>

Values are Mean ± S.D; p<0.05

TBARS (nmoles/mg protein), Catalase (mM of H2O2 consumed/ minute/mg protein), Reduced Glutathione (GSH) (µg/mg protein), Glutathione peroxidase (GPx) (µg of GSH utilized/min /mg protein)
Discussion
It was found that the PZ extract significantly speeds up rats' responses to both the hot plate test and the tail flick test. It is known that centrally acting painkillers raise rats' pain thresholds when they were near heat. There is no doubt that PZ is acting from the center, as shown by the current results. Flavonoids have also been shown to relieve pain by blocking the prostaglandin synthase enzyme, which is the same enzyme that ends up being an antioxidant (Subasini, et al., 2022).

We can see that an extract can reduce inflammation in a living thing by looking at how much edema goes down when we inject a small amount of carrageenan (Subasini, et al., 2013) solution or suspension into the rats' back paw plantar tissues. Most of the time, the carrageenan-induced edema test is used for this type (Uthirapathy, Subasini and Tahsin, Amani (2021)). How much inflammation there is can be seen by how thick the paw is, how heavy it is, or how much mercury (Thenmozhi, S. and Subasini U., 2016) it moves around. Carrageenan is a mixture of polysaccharides composed of galactose units that have been sulfated. It originates from Chondrus crispus, often known as Irish sea moss (Thenmozhi, et al., 2013). There is a change in the amount of a protein-bound marker that leaks into the tissues from the bloodstream.

It's possible that PZ can reduce edema because it stops the release of histamine, 5-hydroxyl tryptamine, and kinins. These chemicals are released when mast cells are activated in the first hour of non-natural paw edema caused by carrageenan (Uthirapathy, S., 2019). Bioactive substances in the extract, including terpenoids, tannins, poly phenols, alkaloids, and flavonoids, reduce the permeability of capillaries to reduce inflammation (Shabi, et al., 2014). Because flavonoids are in PZ extract, it can help reduce inflammation (Thenmozhi, et al., 2012).

Reactive oxygen species (ROS) are continually produced in the majority of tissues and are required for appropriate cell function. The production of ROS may rise in vascular diseases like dyslipidemia, where more ROS may be harmful (Nicholls, D.G, Budd, S.L., 2000). Some of the main ways that living things make ROS are through the oxidation of fatty acids by peroxisomes, the breakdown of foreign compounds by cytochrome P450 microsomal enzymes, aerobic respiration in mitochondria, and the start of phagocytosis by pathogens or lipopolysaccharides and tissue-specific enzymes (Toufektssian, et al., 2001).

The buildup of neutrophils causes swelling to happen. Pro-inflammatory chemicals use and activate these neutrophils in rheumatoid arthritis joints. They damage the joints by releasing granules that contain collagenases and elastase and by generating reactive oxygen species (Krishnamoorthy, et al., 2009; Subasini, et al., 2011). Damage to cartilage is also caused by ROS made by endothelial cells, macrophages, and lymphocytes (Tahsin, et al., 2022). Because of this power, the amount of TBARS found in different body organs, like the liver and kidneys, is higher. This study found a higher level of antioxidants. This could be because the enzymes were working better or because the extract contained bioactive substances that could get rid of free radicals.

When rats were given PZ, their lipid peroxidation level went down. This was because phenolic substances, flavonoids, alkaloids, polyphenols, terpenoids and tannins work together as antioxidants to protect cells from damage caused by free radicals. PZ is an antioxidant that might be able to stop lipid peroxidation, which would mean that fewer lipid peroxidative products would be made. They found that the polyphenol diet stops lipid peroxidation and retains the mitochondrial antioxidant enzyme secure from oxidative stress in their study (Yogeeta, et al., 2006). There are at least a few hydroxyl groups in the phenolic compound that give their electrons to the free radicals. Also, the extract has compounds with a phenolic nucleus and an unsaturated side chain that are mostly there to protect against stress. Flavonoids, catechol-type tannins, terpenoids, phenolic compounds and flavolans are strong antioxidants because they have a lot of hydroxyl groups that interact strongly with proteins (Albert, et al., 2022).

Conclusion
Plumbago zeylanica has several nutrient-dense bioactive substances. The LD50 of Plumbago zeylanica 85% methanolic extract is 4000 mg/kg b.wt. The extract increases hot plate and tail immersion response time, causing analgesia. Carrageenan-induced edema is prevented by 250 mg/kg b.wt. extract. The test extract PZ reduces paw volume and inhibits liver and kidney oxidative stress in CFA-induced rats. It is prevented by increasing the number of antioxidants in the body. The Plumbago zeylanica extract is an effective analgesic, anti-inflammatory, and antioxidant.

Author Contributions
S. U. performed animal study, prepared protocol, analyzed data, and wrote paper.

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Competing financial interests
The authors have no conflict of interest.
References


