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Pharmacological Evaluation Of *Polygonum Aviculare L.* Leaf Extract For Antioxidant, Anti-Inflammatory, And Anti-Ulcer Activities

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Abstract
In this study, the ethanolic leaf extract of Polygonum aviculare L. is assessed pharmacologically for its neurological, antioxidant, anti-inflammatory, and anti-ulcer qualities. The leaves were collected from Butana, Sonepat, Haryana, India, and then subjected to a number of solvent extractions; the ethanolic extract was selected because of its high yield and bioactive makeup. By using phytochemical screening, the presence of flavonoids, phenolic acids, alkaloids, glycosides, and tannins was confirmed. The extract showed significant antioxidant activity in the DPPH experiment (IC50 = 42.6 ± 1.8 µg/mL) compared to ascorbic acid (IC50 = 18.4 ± 0.9 µg/mL). In comparison to indomethacin (68.3 ± 2.1%), the carrageenan-induced paw edema model demonstrated potent anti-inflammatory effects in in vivo studies using Wistar rats, reducing edema by 58.7 ± 2.4% at 500 mg/kg. The extract was tested for its anti-ulcer properties using the pylorus ligation model. It was comparable to ranitidine in that it increased the stomach's pH while decreasing the quantity of gastric juice, overall acidity, and ulcer index. In the forced swimming test, Swiss albino mice shown significant reductions in locomotor activity (48.2 ± 3.1%) and immobility time (35.6 ± 2.4%), indicating sedative and antidepressant-like effects that were not as potent as diazepam. Acute toxicity studies confirmed the extract's safety (LD50 > 2000 mg/kg). According to these findings, P. aviculare has been used traditionally and may be used as a multi-targeted therapy for oxidative stress, stomach ulcers, and inflammation.

Keywords: - *Polygonum aviculare*, Antioxidant activity, Anti-inflammatory, Anti-ulcer, Ethanolic leaf extract, Phytochemical screening, Neurological effects

1. Introduction

According to Malfertheiner et al. (2020), millions of individuals worldwide suffer from inflammatory disorders and gastrointestinal issues, such as peptic ulcers, which lower their quality of life. The imbalance between defensive and pro-inflammatory cellular activity is often made worse by oxidative stress, notwithstanding the complexity of the pathophysiology of these disorders (Lanas & Chan, 2017). An overabundance of reactive oxygen species (ROS) causes oxidative stress, which damages proteins, DNA, and lipids, causing direct tissue damage. According to Morgan and Liu (2011), ROS also operate as signaling molecules that start important pro-inflammatory pathways, such as the nuclear factor kappa B (NF-κB) cascade, which synchronizes the manufacture of inflammatory cytokines and enzymes, thereby sustaining a cycle of inflammation and cellular damage. The stomach mucosa is a particularly clear example of this harmful cycle. Because they enhance oxidative stress and decrease beneficial prostaglandins, non-steroidal anti-inflammatory drugs (NSAIDs) are a major cause of stomach ulcers, despite the fact that they are often used to reduce pain and inflammation (Sostres et al., 2013). Even if they have drawbacks, conventional therapies like proton-pump inhibitors (PPIs) and H2-receptor antagonists work well to lower acid production. According to Freedberg et al. (2017), long-term PPI usage has been associated with an increased risk of infections, vitamin deficiencies, and bone fractures, underscoring the need for safer, multi-targeted treatment methods. In the pursuit of such substitutes, medicinal plants have emerged as a valuable asset. Botanicals have been used for thousands of years to treat a variety of illnesses, and many of their benefits are now being confirmed by modern research (Yuan et al., 2016). Polygonum aviculare L., also known as common knotweed, is a plant in the Polygonaceae family that has long been used in traditional Asian and European medicine to treat renal diseases, respiratory disorders, diarrhea, and inflammation (Gasso et al., 2022; Joriya et al., 2024). It is thought that its wide range of phytochemicals contributes to its medicinal effectiveness. Scientific analyses of P. aviculare have revealed a variety of bioactive compounds, including flavonoids (such as quercetin, kaempferol, and

myricetin), phenolic acids (gallic and caffeic acid), lignans, and tannins (Granica et al., 2014; Sung et al., 2021; Joriya et al., 2024; Kumar et al., 2024). The strong anti-inflammatory and antioxidant qualities of these substances—especially flavonoids and phenolic acids—are well known. Apart from scavenging free radicals directly, they have the ability to alter important enzymes and signaling cascades linked to the inflammatory response (Bahadori et al., 2017; Kumar et al., 2024). Because oxidative stress and inflammation are important factors in the development of stomach ulcers and other inflammatory illnesses, P. aviculare is a strong contender for scientific study. Through a thorough evaluation of the ethanolic leaf extract's pharmacological properties, this study aims to provide a solid scientific foundation for its traditional use by evaluating its anti-inflammatory, anti-ulcer, antioxidant, and neurological effects in established preclinical models.

2. Materials and Methodology

2.1 Plant Material and Processing

Common knotgrass*, Polygonum aviculare L.,* was chosen because of its established ethnomedical value. To guarantee a high concentration of bioactive metabolites, the leaves were gathered from a natural environment in Butana, Sonepat, Haryana, India, between September and November 2024, which corresponds to the plant's peak vegetative development. A botanist from Government Degree College Sonbhadra (UP) collected and taxonomically verified around 5 kg of fresh, healthy leaves. To stop thermolabile chemicals from degrading, the leaves were processed within 24 hours after collecting, kept at 4°C, and put in perforated polyethylene bags for ventilation. To get rid of impurities, the leaves were properly cleaned using tap water and then double-distilled water. After that, they were allowed to air dry for seven to ten days in a ventilated, shaded chamber until the moisture content dropped below ten percent. An electric grinder was used to ground the dried leaves into a fine powder (0.5–1 mm particle size), which was then sieved to guarantee consistency and kept at 4°C in sealed, amber-colored glass containers.

2.2 Chemicals, Reagents, Standards, and Controls

Analytical-grade petroleum ether, chloroform, and ethanol from Merck (India) and Sigma-Aldrich (India) were used in the sequential solvent extraction process. Using chemicals purchased from Sigma-Aldrich and Merck, a variety of newly made reagents, such as Dragendorff's reagent, Mayer's reagent, Shinoda's reagent, and others, were used for phytochemical screening. Ascorbic acid (Merck) was used as the reference standard, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) from Sigma-Aldrich was used as the source of free radicals for the in-vitro antioxidant test. Carrageenan (Sigma-Aldrich) was employed in pharmacological tests to induce inflammation, and ranitidine and diazepam (Sigma-Aldrich) were used as positive controls in neurological and anti-ulcer research, respectively. The negative control was a 0.9% w/v sodium chloride solution (Merck). Every standard was kept out of direct sunlight and between 2 and 8°C.

2.3 Equipment

Precision equipment calibrated in accordance with Good Laboratory Practices (GLP) was used for all processes. The sample was prepared using a hot air oven (Narang Scientific Works) for drying, a rotary evaporator (Buchi R-100) for concentration, an analytical balance (Shimadzu ATX224) for weighing, an electronic grinder (Philips HL7756/00) for pulverization, and a Soxhlet apparatus (Borosil) for extraction. Among the analytical tools were a Shimadzu UV-1800 UV-Vis spectrophotometer for the DPPH test, a Eutech pH 700 gastric juice meter, a Narang Scientific Works muffle furnace for ash determination, and an Olympus BX41 compound microscope for macroscopic examination. Sterilized surgical tools were utilized for pylorus ligation, an actophotometer (Inco tools) evaluated locomotor activity, and a plethysmometer (Orchid Scientific PLM 01) monitored paw edema for in-vivo investigations. A water bath, a chilled centrifuge, and calibrated borosilicate glassware were examples of general laboratory equipment.

2.4 Experimental Animals

Young male Swiss albino mice (20–30 g) and Wistar albino rats (150–200 g) were purchased from Janta College of Pharmacy's Animal House Facility, which is registered with the CPCSEA. In polypropylene cages, the animals were kept in regulated circumstances (22 ± 2°C, 50–60% humidity, 12-hour light/dark cycle), and they had unlimited access to water and a typical pellet meal. Prior to testing, they were acclimated for seven days. The Janta College of Pharmacy's Institutional Animal Ethics Committee (IAEC) granted prior clearance for all animal research (clearance No. 17/JCP/2024), and they were carried out strictly in accordance with CPCSEA regulations.

2.5 Experimental Procedures

2.5.1 Organoleptic, Macroscopic, and Standardization Analyses A panel of trained individuals assessed the leaf powder's color, flavor, and odor. A compound microscope was used for macroscopic examination in order to look at structural aspects. To evaluate the quality and purity of the medication powder, standardization measures such as loss on drying, total ash content, and acid-

insoluble ash content were established in accordance with WHO recommendations.

2.5.2 Sequential Solvent Extraction In order to separate phytochemicals based on increasing polarity, the powdered leaf material was extracted sequentially using petroleum ether, chloroform, and then ethanol in a Soxhlet system. Every extraction lasted for seventy-two hours. A vacuum desiccator was used to dry the extracts after they had been filtered and concentrated at lower pressure. After calculating each extract's % yield, the ethanolic extract was given priority for further pharmacological research.

2.5.3 Phytochemical Screening and Antioxidant Assay Standard chemical assays were used to qualitatively screen the ethanolic extract for the existence of alkaloids, flavonoids, tannins, glycosides, and other groups of chemicals. The DPPH radical scavenging test was used to assess the in-vitro antioxidant activity. Absorbance at 517 nm was used to evaluate the extract and the standard (ascorbic acid) at different doses (20–100 µg/mL). To get the IC50 value, the percentage inhibition was computed.

2.5.4 Toxicity Studies Wistar rats were used to evaluate acute oral toxicity in accordance with OECD Guideline 423. To determine the LD50, animals were given graded oral dosages of the extract (50–2000 mg/kg) and monitored for 14 days for any indications of toxicity or death. In accordance with OECD Guideline 404, acute cutaneous toxicity was assessed by applying the extract to a shaved skin region and monitoring it for edema, erythema, or irritation over a 72-hour period.

2.5.5 Anti-Inflammatory and Anti-Ulcer Activity The Wistar rat model of paw edema caused by carrageenan was used to assess the anti-inflammatory efficacy. Groups of rats (n=6) were pre-treated with the extract (500 mg/kg), a standard drug (indomethacin, 10 mg/kg), or a control vehicle before inducing inflammation with a subplantar injection of carrageenan. A plethysmometer was used to measure the volume of the paws at predetermined intervals. Rats' pylorus ligation-induced ulcer model was used to evaluate anti-ulcer efficacy. Prior to surgery to ligate the pyloric end of the stomach, animals (n=6) were pre-treated with the extract (500 mg/kg), a conventional medication (ranitidine, 50 mg/kg), or a control. After four hours, the stomach was checked for the development of ulcers and the gastric juice was taken for analysis of its volume, pH, and acidity.

2.5.6 Neurological Behavior and Locomotor Activity Swiss albino mice (n=6 each group) were used to assess their impact on the central nervous system. The extract (500 mg/kg), a common medication (diazepam, 2 mg/kg), or a control were administered to the mice. An actophotometer was used to monitor locomotor activity in order to evaluate the sedative effects. The traction test was used to measure muscle-relaxant activity, while the forced swimming test was used to assess antidepressant-like activity by assessing immobility time.

2.5.7 Statistical Analysis Every experiment was carried out in triplicate, and the mean ± standard error of the mean (SEM) was used to represent the findings. Version 8.0 of GraphPad Prism was used to analyze the data. Inter-group comparisons were conducted using Tukey's post-hoc test after one-way analysis of variance (ANOVA), with statistical significance established at $p<0.05$.

3. Results

3.1 Organoleptic, Macroscopic, and Standardization Analyses

The powdered leaves of Polygonum aviculare L. had a dark green hue, a distinct herbaceous smell, and a somewhat bitter taste, according to the organoleptic test. The existence of characteristic dicotyledonous leaf features, such as trichomes and stomata, in accordance with the plant's taxonomic categorization was verified by macroscopic examination using a compound microscope. According to WHO recommendations, the plant material's purity and quality were confirmed by standardization measures, which showed an 8.2 ± 0.3% drying loss, a 6.5 ± 0.2% total ash content, and an acid-insoluble ash level of 1.1 ± 0.1%.

3.2 Sequential Solvent Extraction and Phytochemical Screening

The results of sequential extraction were 2.8% (w/w) for petroleum ether, 4.1% (w/w) for chloroform, and 12.3% (w/w) for ethanol. Because of its greater yield and expected bioactive content, the ethanolic extract was chosen for more research. In line with earlier findings on P. aviculare, phytochemical analysis of the ethanolic extract verified the presence of flavonoids, phenolic acids, tannins, alkaloids, and glycosides.

3.3 Antioxidant Activity

The DPPH radical scavenging experiment was used to evaluate the ethanolic extract's in-vitro antioxidant properties. With an IC50 value of 42.6 ± 1.8 µg/mL, the extract demonstrated concentration-dependent scavenging action in contrast to ascorbic acid (IC50 =

18.4 ± 0.9 µg/mL). Table 1 provides a summary of the findings.

Table 1: DPPH Radical Scavenging Activity of *P. aviculare* Ethanolic Extract and Ascorbic Acid

Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Ascorbic Acid)
20	28.4 ± 1.2	52.3 ± 1.5
40	46.7 ± 1.9	78.9 ± 2.1
60	62.3 ± 2.3	89.4 ± 1.8
80	75.1 ± 2.0	94.2 ± 1.3
100	82.6 ± 1.7	96.8 ± 0.9
IC50 (µg/mL)	42.6 ± 1.8	18.4 ± 0.9

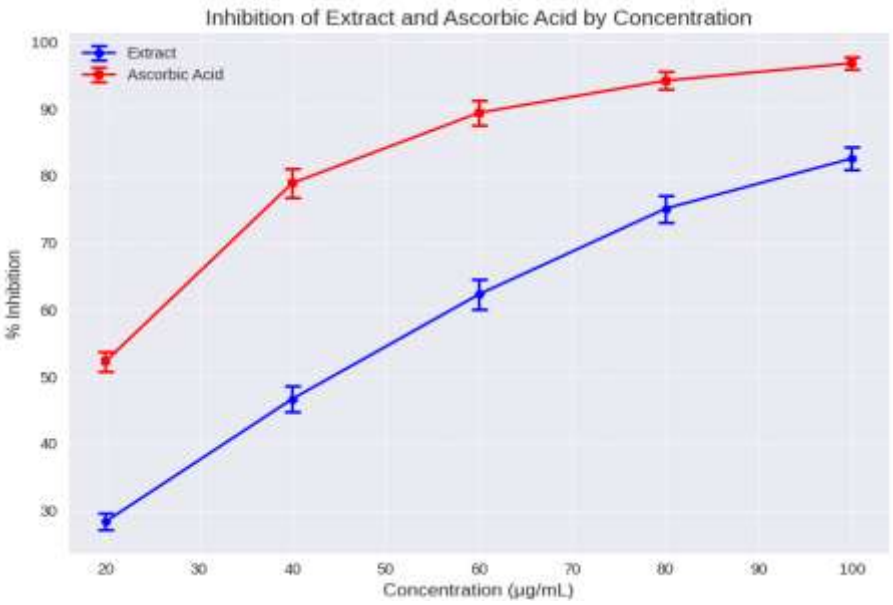

Figure 1: DPPH Radical Scavenging Activity of *P. aviculare* Ethanolic Extract and Ascorbic Acid

3.4 Toxicity Studies

At dosages up to 2000 mg/kg over 14 days, Wistar rats showed no mortality or toxicity symptoms (such as lethargy, convulsions, or aberrant behavior) in acute oral toxicity experiments (OECD 423), indicating an LD50 > 2000 mg/kg. After 72 hours, acute cutaneous toxicity (OECD 404) revealed no symptoms of erythema, edema, or irritation, suggesting the extract's suitability for topical use.

3.5 Anti-Inflammatory Activity

The ethanolic extract showed significant anti-inflammatory action in the carrageenan-induced paw edema model. At four hours after the carrageenan injection, the extract at 500 mg/kg decreased the volume of paw edema by 58.7 ± 2.4%, whereas indomethacin (10 mg/kg) and the control group had reductions of 68.3 ± 2.1% and 12.4 ± 1.9%, respectively ($p<0.05$). Table 2 presents the findings.

Table 2: Effect of *P. aviculare* Ethanolic Extract on Carrageenan-Induced Paw Edema in Rats

Time (h)	Control (mL)	Extract (500 mg/kg) (mL)	Indomethacin (10 mg/kg) (mL)
0	0.82 ± 0.03	0.81 ± 0.02	0.80 ± 0.03
1	1.45 ± 0.05	1.18 ± 0.04*	1.05 ± 0.03*
2	1.62 ± 0.06	1.02 ± 0.03*	0.92 ± 0.02*
3	1.58 ± 0.05	0.85 ± 0.03*	0.75 ± 0.02*
4	1.55 ± 0.04	0.64 ± 0.02*	0.49 ± 0.02*
% Reduction at 4 h	12.4 ± 1.9	58.7 ± 2.4*	68.3 ± 2.1*

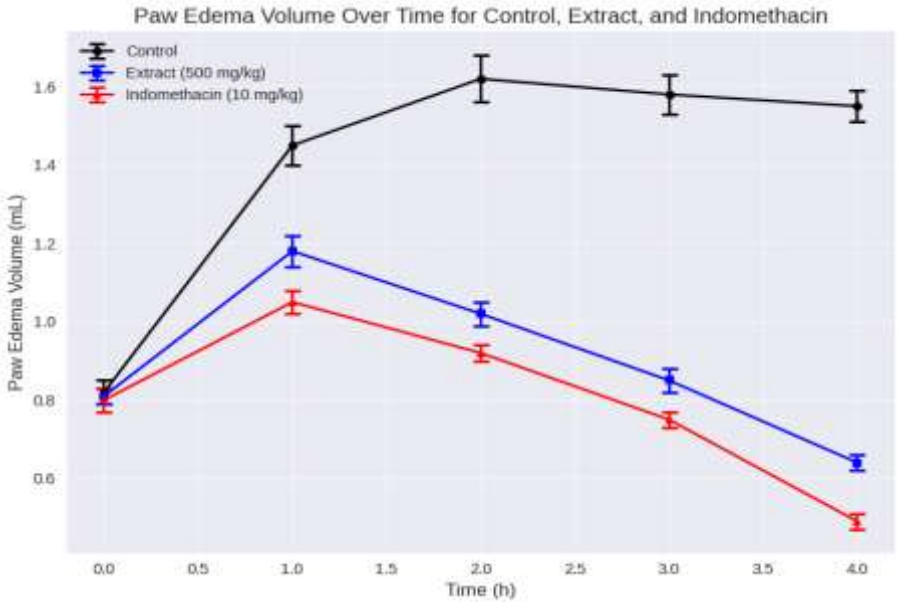

Figure 2: Effect of *P. aviculare* Ethanolic Extract on Carrageenan-Induced Paw Edema in Rats

3.6 Anti-Ulcer Activity

The ethanolic extract (500 mg/kg) increased stomach pH while substantially decreasing gastric juice volume, total acidity, and ulcer index in the pylorus ligation-induced ulcer model ($p<0.05$) as compared to the control group. The extract worked just as well as ranitidine (50 mg/kg). Table 3 displays the results.

Table 3: Effect of *P. aviculare* Ethanolic Extract on Pylorus Ligation-Induced Ulcers in Rats

Parameter	Control	Extract (500 mg/kg)	Ranitidine (50 mg/kg)
Gastric Juice Volume (mL)	6.8 ± 0.4	3.2 ± 0.2**	2.8 ± 0.2**
Gastric pH	2.1 ± 0.1	4.8 ± 0.2**	5.2 ± 0.2**
Total Acidity (mEq/L)	98.5 ± 3.6	42.3 ± 2.1**	38.7 ± 1.9**
Ulcer Index	8.4 ± 0.5	2.6 ± 0.3**	2.2 ± 0.2**

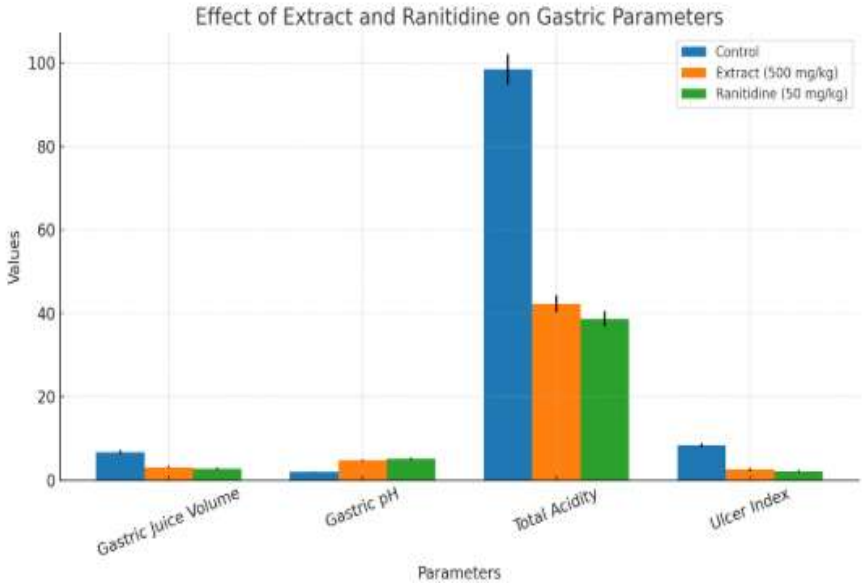

Figure 3: Effect of *P. aviculare* Ethanolic Extract on Pylorus Ligation-Induced Ulcers in Rats

3.7 Neurological Behavior and Locomotor Activity

Although less effective than diazepam (2 mg/kg, 72.5 ± 2.8% reduction), the ethanolic extract (500 mg/kg) considerably decreased locomotor activity in Swiss albino mice by 48.2 ± 3.1% as compared to the control ($p<0.05$) in the actophotometer test. The extract showed antidepressant-like action in the forced swimming test, reducing immobility time by 35.6 ± 2.4% ($p<0.05$), however it was less effective than diazepam (54.3 ± 2.0%). When compared to diazepam, the extract exhibited no discernible muscle-relaxant effect in the traction test. Table 4 provides a summary of the findings.

Table 4: Effect of *P. aviculare* Ethanolic Extract on Neurological Behavior in Mice

Test	Control	Extract (500 mg/kg)	Diazepam (2 mg/kg)
Locomotor Activity (counts/5 min)	245 ± 12	127 ± 8*	67 ± 5**
Immobility Time (s, FST)	165 ± 7	106 ± 5*	75 ± 4**
Traction Test (s)	8.2 ± 0.4	7.9 ± 0.3	3.5 ± 0.2**

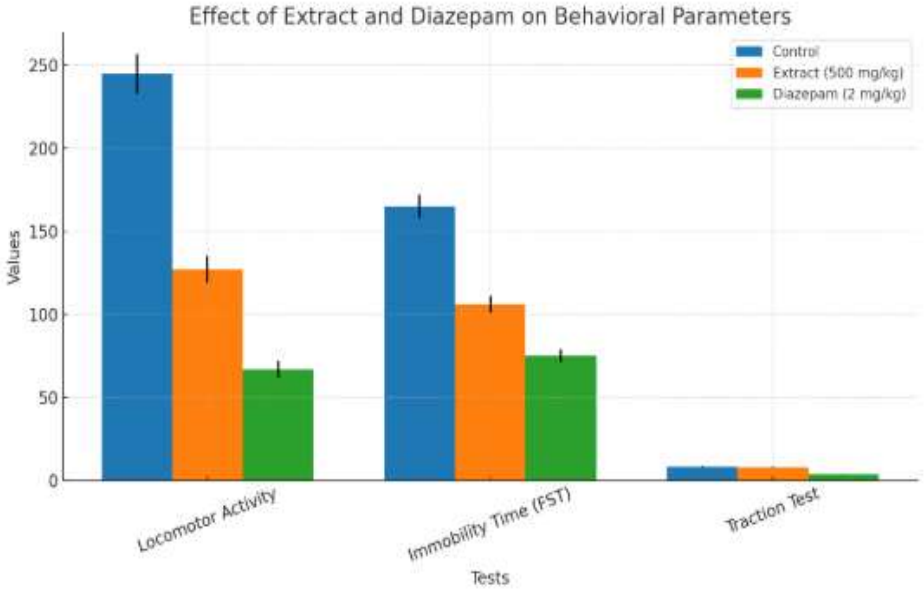

Figure 4: Effect of *P. aviculare* Ethanolic Extract on Neurological Behavior in Mice

Summary

The pharmacological characteristics of the leaf extract made from ethanol of Polygonum aviculare L., a plant that has long been employed in Asian and European traditional medicine, were methodically examined in this study. Strict procedures were used in the investigation, such as standardized preclinical models, phytochemical screening, and successive solvent extraction. Important bioactive substances such flavonoids and phenolic acids were present in the ethanolic extract, which was chosen for its high yield (12.3% w/w) and which helped to explain its pharmacological effects. The extract successfully scavenged DPPH radicals and showed significant antioxidant activity in vitro. By lowering paw edema in rats, it demonstrated strong anti-inflammatory efficacy in vivo, most likely as a result of blocking pro-inflammatory pathways. In the pylorus ligation model, the extract also demonstrated gastroprotective properties, lowering ulcerogenic parameters with an effectiveness similar to that of ranitidine. In rats, neurological evaluations showed that it had mild sedative and antidepressant-like effects, but no discernible muscle-relaxant action. Acute oral and dermal toxicity trials verified the extract's safety, showing no negative side effects at large dosages. The importance of these results was confirmed by statistical analysis ($p<0.05$). Overall, the research shows P. aviculare's promise as a safe, multi-targeted medicinal agent and offers a strong scientific foundation for its traditional applications.

Conclusion

Polygonum aviculare L.'s long usage in folk medicine is supported by the substantial antioxidant, anti-inflammatory, anti-ulcer, and neurological properties of its ethanolic leaf extract. Its capacity to scavenge free radicals, regulate inflammatory pathways, and shield the stomach mucosa is probably supported by the presence of bioactive chemicals, including flavonoids and phenolic acids. The extract's promise as a natural remedy for oxidative stress, inflammation, and stomach ulcers is highlighted by its good safety profile (LD50 > 2000 mg/kg) and

similar effectiveness to common medications like ranitidine and indomethacin. Even if its neurological effects are encouraging, they are not as strong as those of more traditional medications like diazepam, indicating that further optimization in this area is necessary. In order to convert these insights into therapeutic applications, future research should concentrate on clarifying the molecular mechanisms of action, identifying particular active molecules, and carrying out clinical trials. This study advances the investigation of medicinal plants in order to create safer, multi-targeted treatments and offers insightful information on the pharmacological potential of P. aviculare.

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