Study of the Analgesic Properties of Hydro-Alcoholic Extract of *Aristolochia Bracteolata* Leaves and *Peganum Harmala Seeds.*

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ABSTRACT

Pain is a complex experience of physiology and sensation which serves as protection for the body. Therefore, it signals potential damage to tissue and; actual damage. Conventional analgesic drugs include non-steroidal ant-inflammatory drugs (NSAIDs) and opioids with which pain is managed. However, long-term use has been associated with adverse effects such as gastrointestinal toxicity, kidney impairment, addiction, and tolerance. This necessitates the exploration of plant-based alternatives that are safe for use in pain relief. This study evaluates the analgesic activity of *Aristolochia bracteolata* and *Peganum harmala* using Eddy's Hot Plate method that had previously proven a good model for assessing centrally mediated analgesic activity in rodents. The plant extracts were prepared by hydroalcoholic Soxhlet extraction, and the phytochemical profile of those extracts was evaluated. Swiss albino mice and Wistar rats were treated with the extracts at 200 mg and 400 mg of extract per kg body weight, respectively, and the response latency to thermal stimuli was recorded. The results demonstrated a concentration-dependent significant elevation in latency time post-administration, which strongly indicates analgesic activity comparable to that of the standard drug pethidine. The exhibited analgesic effects are likely caused by their active constituent’s bioactive components, such as flavonoids, alkaloids, and β-carbolines, which are known to control inflammatory mediators and the pathways of neurotransmitter transmission. This observation gives a scientific support to the reason for possible benefits of pain relief in traditional

use of *Aristolochia bracteolata* and *Peganum harmala*. However, further studies will be needed to establish their detailed mechanisms and possible clinical applications.

Keywords: Pain, *Aristolochia bracteolata, Peganum harmala*, Analgesic activity, Eddy's Hot Plate, Phytochemicals, Traditional Medicine

Introduction

Pain is a multifaceted physiological and sensory event that is a protective mechanism that informs the body of possible or actual tissue damage [1]. Pain is divided into acute and chronic pain depending on duration and etiology. Acute pain is a transient reaction to injury, whereas chronic pain is an enduring phenomenon following normal healing and has a significant impact on mobility, mental health, and quality of life [2]. Pain may also be divided into nociceptive and neuropathic pain. Nociceptive pain is caused by the stimulation of nociceptors from mechanical, thermal, or chemical sources, which are usually seen in inflammation or tissue damage [3]. Neuropathic pain, however, is caused by damage or disease in the nervous system and is usually unresponsive to standard analgesics [4]. To effectively control pain, non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are the major pharmacologic agents employed [5].

NSAIDs work by blocking cyclooxygenase (COX) enzymes, which are involved in prostaglandin production [6]. While effective, chronic use of NSAIDs carries significant adverse effects in the form of gastrointestinal ulcers, renal toxicity, and heightened cardiovascular risk [7]. Opioids, which exert their effects on the central nervous system (CNS) opioid receptors, are highly effective in causing analgesia but are associated with serious hazards, including tolerance, dependence, addiction, and respiratory depression [8]. In view of these constraints, there is a pressing need to investigate safer and more effective natural alternatives for analgesia. For centuries, medicinal plants have been integral to pain control in traditional medicine systems like Ayurveda, Traditional Chinese Medicine (TCM), and Unani [9]. These medicinal plants bear a wide array of bioactive phytoconstituents, such as alkaloids, flavonoids, terpenoids, and phenolics, with analgesic properties by way of modulating inflammatory mediators, neurotransmitters, and nociceptive pathways [10].

Since they are not toxic, low in cost, and readily available, plant therapies are increasingly studied as pain management alternatives to artificial drugs [11]. Out of these, *Aristolochia bracteolata* and *Peganum harmala* have also been traditionally prescribed for their analgesic and anti-inflammatory activities [12]. *Aristolochia bracteolata*, better known as Bracted Dutchman's Pipe or Worm Killer, consists of aristolochic acids, flavonoids, and alkaloids, which have been proposed to mediate nociceptive and inflammatory processes [13]. Likewise, *Peganum harmala*, commonly known as Syrian Rue, is rich in β-carboline alkaloids, like harmine and harmaline, which affect serotonergic and dopaminergic systems and have been found to show analgesic activity [14].

In order to scientifically establish the analgesic potential of these plants, the current study employs the Eddy's Hot Plate method, a commonly used experimental model for assessing centrally mediated pain responses [15]. In this test, animals are placed on a heated surface, and the time (latency period) to the onset of pain-related behaviors (e.g., paw licking or jumping) is measured [16]. Elevated reaction time after plant extract administration indicates an analgesic effect, which would be expected to be mediated through central pain modulation [17]. This study has the objective of presenting scientific evidence of the traditional use of *Aristolochia bracteolata* and *Peganum harmala* for pain management [18]. Through the discovery of plant-derived analgesics, this study may also help to advance the development of safer, more effective, and affordable alternatives to traditional pain medication, reducing the dangers posed by synthetic medicine [19].

Materials and Methods

Plant Collection and Extraction

Leaves of *Aristolochia bracteolata* and seeds of *Peganum harmala* were obtained from a local market and were identified at Janani Organics, Hubali. Dry plant material was coarsely powdered and underwent Soxhlet extraction with a hydroalcoholic solvent (70% ethanol and 30% water). The process of extraction was performed for 24 hours in order to facilitate the effective recovery of bioactive molecules. The extract was filtered and dried completely. The dried extract was measured and stored in an air-tight jar at 5°C until later analysis [20]. The preliminary phytochemical screening of the hydroalcoholic extracts was done for the determination of

alkaloids, flavonoids, saponins, tannins, steroids, and secondary metabolites through general qualitative tests.

Animals and Ethical Approval

Swiss albino mice (20–40 g) and female Wistar albino rats (180–220 g) were obtained from a local vendor and were kept at 25 ± 2°C with a relative humidity of 45% to 55% under standard environmental conditions (12-hour light: 12-hour dark cycle) in the animal house. The animals had unrestricted access to food and water during the study. The experiment was performed following CPCSEA guidelines, and approval from the Institutional Animal Ethics Committee (IAEC) was obtained [21].

Experimental Design

Acute toxicity study of hydroalcoholic extract of *Aristolochia bracteolata* leaves & Seeds of *Peganum harmala* as per OECD guideline 425.

The animals were randomly divided into five groups, each comprising six rats:

Group I: received of normal saline (control group)

Group II: Pain sensations were induced by hot plate method

Group III: received pethidine 30 mg/kg (standard group) i.p. orally + Pain sensations were induced by hot plate method

Group IV: received extract of *Aristolochia bracteolate* 200mg/kg orally + Pain sensations were induced by hot plate method

Group V: received extract of *Aristolochia bracteolate* 400mg/kg orally + Pain sensations were induced by hot plate method

Group VI: received extract of *Peganum harmala* 200mg/kg orally + Pain sensations were induced by hot plate method

Group VII: received extract of *Peganum harmala* 400mg/kg orally + Pain sensations were induced by hot plate method

Eddy's Hot Plate Method

Analgesic activity was measured by the hot plate test of Eddy and Leimbach in rats [22]. For this experiment, the hot plate equipment was kept at 55 ± 0.1°C. Rats were put into an acrylic cylinder (20 cm diameter) on the heated surface, and the time between being placed and licking their hind paws or jumping was measured as the response latency. A cut-off of 60 s was employed for preventing tissue damage. The latency to respond was measured prior to (0 min) and 30 min, 60 min, and 120 min after oral drug administration. Analgesia was operationalized as latency extension without hind limb flick or lick or jumping. Animals with latencies > 60 s at 0 min were excluded. For each species, the % maximum possible effect (% MPE) was computed using the

formula: %MPE= (post-drug latency−Pre-drug latency20−Pre-drug latency) ×100\%

Results

The results of hot plate test revealed that the *Aristolochia bracteolate* and *Peganum harmala* significantly ($p<0.05$, $p<0.01$) increased the latency time after 30 mins compared to control group, while both extracts exhibited more significant ($p< 0.001$) increase in latency time after 60 mins. The significant increase in latency time was observed up to 120 mins. in all extracts. The findings revealed analgesic action of the both extracts *Aristolochia bracteolata* and *Peganum harmala* at both doses i.e. 200 mg/kg p.o. and 400 mg/kg p.o. The reference pethidine produced more marked ($p< 0.001$) prolongation in latency time at all study intervals.

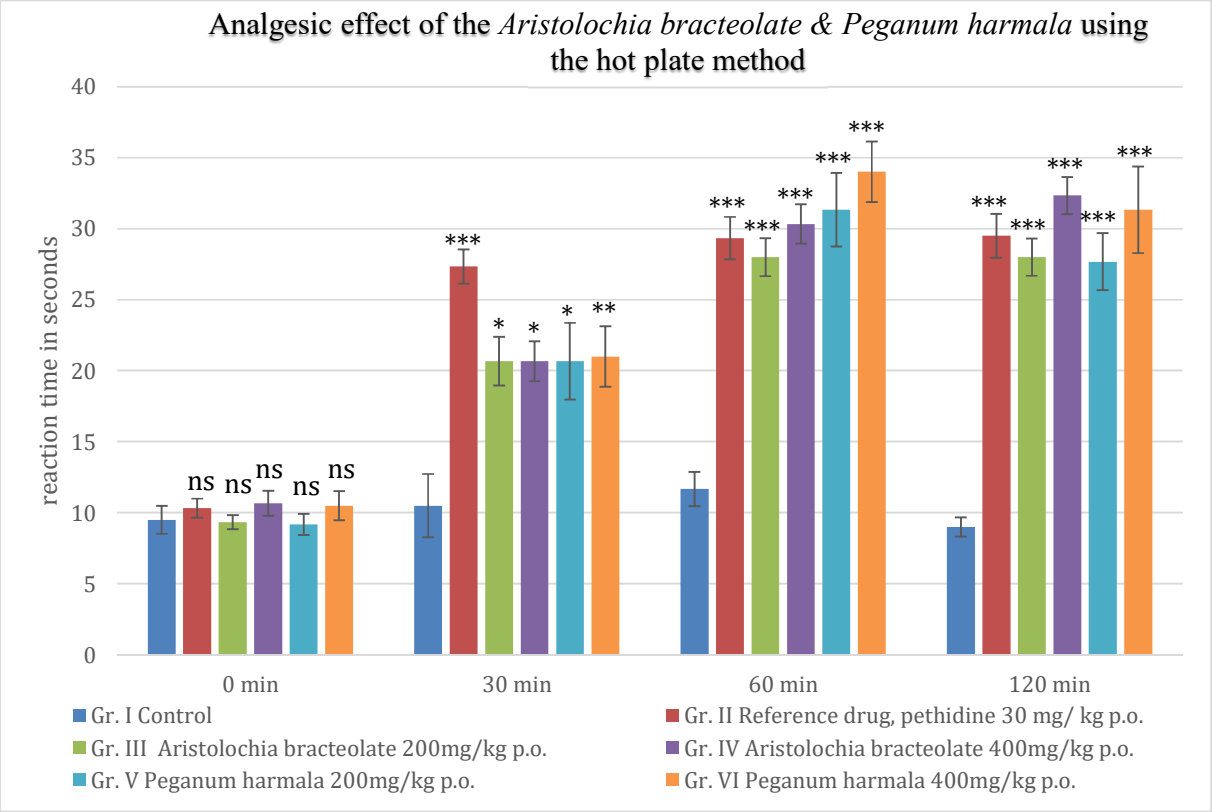

Figure 1: Analgesic effect of the *Aristolochia bracteolata & Peganum harmala* using the hot plate method

Discussion

Pain is a multifaceted physiological response mediated through nociceptive pathways that include both peripheral and central mechanisms. The hot plate test is a reliable method to evaluate centrally acting analgesics since it determines the latency to thermal stimulation, which is largely mediated through supraspinal processing and opioid receptors [23,24]. In this study, the analgesic activity of *Aristolochia bracteolata* and *Peganum harmala* was tested using the hot plate test in rats, and the study showed a significant analgesic effect.

The results revealed that both *Peganum harmala* and *Aristolochia bracteolata* increased the latency period upon thermal stimuli significantly, reflecting an analgesic activity. The rise in latency was significant as early as 30 minutes after administration ($p < 0.05$, $p < 0.01$), with the effect being more intense at 60 minutes ($p < 0.001$). This effect was maintained up to 120 minutes,

indicating prolonged analgesic activity of both extracts. The dose-response also indicates their action in pain modulation with both 200 mg/kg and 400 mg/kg inducing significant effects. Nonetheless, the 400 mg/kg dose was more potent, indicating increased efficacy at higher doses.

The standard drug pethidine, a nonsteroidal anti-inflammatory drug (NSAID) whose effects are peripheral and central analgesic, registered a highly significant ($p < 0.001$) prolongation of the latency time at all intervals observed. This indicates that the mechanism of pethidine, whose action is mainly through cyclooxygenase (COX) inhibition and suppression of prostaglandin, is very effective in pain mitigation [25]. In contrast to the control, *Aristolochia bracteolata* and *Peganum harmala* also exhibited significant analgesic activities, suggesting that they can be used as potential alternative analgesics.

The analgesic activity reported in this study can be explained by the bioactive compounds of *Aristolochia bracteolata* and *Peganum harmala*. Earlier research has shown that *Aristolochia bracteolata* is rich in flavonoids, alkaloids, and terpenoids, which have been associated with analgesic and anti-inflammatory activities [26]. Flavonoids, specifically, are known to influence pain perception by suppressing inflammatory mediators and binding to opioid receptors [27]. Likewise, *Peganum harmala* contains β-carboline alkaloids like harmaline and harmine, which have been shown to possess analgesic effects by influencing neurotransmitter systems like serotonin and dopamine [28].

The large rise in pain threshold indicates that both extracts might have multiple mechanisms of analgesia, such as opioid receptor modulation and prevention of inflammatory mediators. Since the hot plate test is mainly designed to assess centrally mediated analgesia, it can be speculated that these extracts may act through central nervous system mechanisms rather than strictly peripheral anti-inflammatory action.

Conclusion

The current study brings to light the remarkable analgesic potential of *Aristolochia bracteolata* and *Peganum harmala*, as reflected by increased hot plate latency. Both extracts displayed dose-dependent analgesia with a greater intensity and duration at 400 mg/kg. Although pethidine was the most potent, *Aristolochia bracteolata* and *Peganum harmala* had similar analgesic activity,

validating their folk use as analgesics. Additional studies are needed to clarify the precise mechanisms through which they exert their analgesic effects and assess their potential for clinical use.

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