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EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF *ARGYREIA NERVOSA* AERIAL PARTS EXTRACT

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ABSTRACT
This study investigates the analgesic and anti-inflammatory effects of aqueous and ethanol extracts of *Argyreia Nervosa* aerial parts. Given the long-term adverse effects associated with conventional analgesics, NSAIDs and corticosteroids the search for safer alternatives remains critical. The extracts were prepared through cold maceration and Soxhlet methods, followed by phytochemical screening and acute oral toxicity assessment to establish safe dosage levels. Analgesic activity was evaluated using the Eddy's hot plate method, while anti-inflammatory effects were assessed through histamine-induced paw oedema in rats. Results indicated that both extracts exhibited significant analgesic effects at a dose of 400 mg/kg, as well as marked reductions in paw volume in the respective models. This study concludes that *Argyreia Nervosa* aerial parts possess considerable analgesic and anti-inflammatory properties, suggesting its potential as a safer therapeutic agent in managing pain inflammation.

KEYWORDS: *Argyreia Nervosa*, Analgesic, Anti-inflammatory, Eddy's hot plate, Plethysmograph.

INTRODUCTION
Medicinal plants have been used for centuries as remedies for human diseases, and over 25% of pharmaceuticals today are derived from natural products. Interest in natural product research remains strong due to therapeutic needs, biological activities of secondary metabolites, bioactive properties of natural products, development of new techniques, and success of herbal drugs in the global market.[1-2] In India, around 20,000 medicinal plant species have been recorded, but traditional communities use only 800 plant species for various diseases.[3] To improve the quality of Ayurvedic medicines, good manufacturing practice guidelines, medicinal plant boards, herbal gardens, and institutes like NIPER, NBRI, CIMAP, and CDRI play pivotal roles.[4] This study aims to explore the analgesic and anti-inflammatory activities of *Argyreia Nervosa* aerial parts extracts in experimental animals like mice and rats.

Physiology of pain
Pain is a human primate instinct that triggers distressing sensations and emotional experiences linked to tissue damage. It activates receptors in primary afferent fibers, including the unmyelinated C-fiber and myelinated Aσ-fiber, which are activated when a potential noxious stimulus is detected. The brain requires sensory events to detect pain and respond. Pain perception involves three stages: pain sensitivity, transmission from the periphery to the dorsal horn via the peripheral nervous system, and transmission to the higher brain via the central nervous system. Pain affects various body regions and can be classified into three major classes: nociceptive pain, neuropathic pain, and inflammatory pain.[5]

Mechanism of pain
The basic pain mechanism involves three events: transduction, transmission, and modulation. Transduction converts stimulus events into chemical tissue, electrical events in neurons, and synaptic cleft events. Transmission transmits these electrical events along neuronal pathways, while modulation occurs at all levels of nociceptive pathways. This process initiates and completes the pain pathway, allowing us to feel the painful sensation triggered by the stimulus.[6]

Inflammation
Inflammation is the immune system's response to harmful stimuli, such as pathogens or damage[7], and is crucial for health. It minimizes injury or infection, restores tissue homeostasis, and resolves acute inflammation. However, uncontrolled inflammation can lead to chronic diseases.[8] Inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function. Pathogenic factors can induce inflammation,

and the body initiates a chemical signaling cascade to heal affected tissues.[9]

NF-κB pathway

The NF-κB pathway is a crucial transcription factor involved in inflammatory, immune response, survival, and apoptosis processes.[10] It is induced by various stimuli and is activated by IκB kinase (IKK).[11] IKK regulates NF-κB pathway activation through IκB phosphorylation[12], leading to the release of NF-κB for gene transcription and nuclear translocation.[13]

Mediators and biomarkers of inflammation

The discovery of inflammatory mediators and sensitive biomarkers has improved our understanding of inflammation and its role in pathology.[14] These include reactive oxygen species, DNA adducts, cytokines, acute-phase proteins, prostaglandins, COX-related metabolites, inflammation-related growth factors, and major immune cell types.[15-16]

MATERIALS AND METHODS

Collection and authentication of plant material: The Aerial parts of *Argyreia nervosa* were obtained from a reputable botanist in the district of Washim, Maharashtra, and the plant material was authenticated by Dr. Pramod Kumar Professor and the Head of the Department of Pharmacognosy at the V. L. College of Pharmacy, Raichur. (NETPC-6/2023-24 is the number for the herbarium specimen.) The collected plant material was washed with water, and samples were then air-dried with a dehumidifier at room temperature. The samples were put into a sealed plastic container after being mechanically ground to powder.

Preparation of aqueous extract

Method of maceration: In this procedure, the entire or coarsely powdered crude medication was put in a stoppered container (1:7 ratio) with the solvent and let to stand at room temperature for at least 7 days while being frequently stirred to dissolve the soluble material. Then, the combined liquids were clarified by filtration or decantation after standing. The mixture is then strained, the marc (the moist solid material) was pressed, and the mixture was clarified.[17-18]

Preparation of alcoholic extract

Soxhlet extraction: When there are insoluble contaminants present and the solid sample has a limited solubility in a solvent, Soxhlet extraction has historically been used. The main chamber of the Soxhlet extractor was filled with a porous thimble containing a solid sample. The extraction cycle was frequently repeated by refluxing the solvent through the thimble using a condenser and a syphon side arm. Soxhlet extraction is a reliable, time-tested method that allows for unattended extraction. Unfortunately, it necessitates a protracted extraction period and substantial solvent use. The solvent was reconstituted in a CE separation solution after evaporation and then injected into the capillary because the extract volume is significantly bigger than a usual injection volume for CE.[19]

Experimental animals

Inbred male and female Wistar albino strain rats(150-200g) and mice (20-25g) were received from animal house of institute. The project was approved by Institutional Animal Ethical Committee (IAEC) of CCSEA (Committee For Control and Supervision of Experiments on Animals). The project proposal has passed the ethics clearance from IAEC Reference No: IAEC/NETPC/2/2023 dated on 8/08/2023.

All the rats were housed in polypropylene cages and fed with standard rodent pellet feed and water were provided ad libitum throughout experimentation period. They were allowed to access 12:12hr Light: day cycle under standard laboratory conditions temperature 24-28^{0}C, Relative humidity 60 to 70 %. The animals were acclimatized to laboratory condition for a period of one week prior to initiation of study. Female rats were housed separately and used at the end of the study.

Preliminary phytochemical analysis[20]

Standard phytochemical screening test was carried out to detect the presence of secondary metabolites to relate the analgesic and anti-inflammatory of *argyreia nervosa* aerial parts extract with the presence or absence of these active constituents. Thus, the test for alkaloids, saponins, flavonoids, phenols, steroid, anthraquinone, glycosides and tannins were performed using standard test procedures.

ANALGESIC ACTIVITY[21]

Analgesia is an ill-defined, unpleasant sensation usually evoke by an external or internal noxious stimulus. Analgesic a drug that selectively relives by acting in the CNS or Peripheral pain mechanisms without significant by altering consciousness.

Eddy's hot plate method

Eddy's hot plate method is used for screening of central analgesic activity. In this method heat is used as a source of pain Animal is placed individually on the hot plate, maintained at constant temperature (55 °C ± 1 °C). The reaction time i.e. Time taken by the animal to lick its hind paw or to leap out planning it on the hot plate is taken as the reaction of painful stimuli Analgesics increase the reaction time. This method was first described by Eddy and Limbach.

Procedure: Albino mice of either sex were selected and divided into six groups containing four animals in each group. These animals were tested for 18hrs prior to the experiment. Animal of Group-I considered as control which were administered with 5%gum acacia. Whereas Group-2 was treated with standard drug i.e., Pentazocine (10mg/kg) and was considered as standard group. Remaining four groups were treated with medium and high dose of body weight, aqueous and ethanolic aerial

parts extracts of *Argyreia Nervosa* respectively. The reaction time for each group was recorded at 0, 30, 60, 90 and 120 minutes after the administration, standard drug, aqueous and ethanolic extract of *Argyreia Nervosa* aerial parts.

ANTI-INFLAMMATORY ACTIVITY

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical agents. It is body's response for tissue repair and it is triggered by the release of chemical mediators from the injured tissue and migrating cells. The specific chemical mediators vary with the type of inflammatory process and include animals such as histamine, serotonin and lipid such as prostaglandins and small peptides such as resins. The acute inflammatory response has three main functions

1) The affected area occupied by a transient material called the acute inflammatory exudate. The exudate carries proteins, fluid and cells from local blood vessels into the damaged area to mediate local defences.

2) If an inductive causative agent (eg. bacteria) is present in the damaged area, destroyed and eliminated by components of the exudate.

3) The damaged tissue can be broken down and partially liquified and the debris removed from the site of damage.

Histamine induced rat paw oedema model[22]

Procedure: Albino rats of either sex weighing 150-200gms were selected, they were maintained on standard relet diet and free access to water. The animals were divided into six groups each having four animals. The various groups were treated with standard drug, aqueous and ethanolic extract of *Argyreia Nervosa*. The normal control, diclofenac, aqueous and ethanolic extract were administered to the rats 30 min before the injection of 0.1 ml of 1% histamine suspension in normal saline. A no. 26 gauge needle was used to inject the histamine suspension into the sub plantar region of the left hind paw and the right hind paw served as reference. Immediately thereafter the oedema volumes of the injected paws were measured plethismographically by mercury displacement method. For comparison purpose the volume of oedema at various prefixed times intervals was measured. The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated percentage reduction in oedema volume was calculated by using the formula

$$\text{Percentage} = \frac{V0-V1}{V} \text{ X } 100$$

Where,

Vo-Volume of the paw of control at time "t"

V_1-Volume of the paw of drug treated at the time 't' From the obtained data, the mean oedema volume and the percentage reduction in oedema was calculated.

RESULTS AND DISCUSSION

Table 1: Results of preliminary phytochemical analysis.

Sl. No.	Tests	Aqueous Extract	Ethanolic Extract
1	**Alkaloids**		
	Dragendroff's test	+ve	+ve
	Mayer's test	+ve	+ve
	Hager's test	+ve	+ve
	Wagner's test	+ve	+ve
2	**Carbohydrate**		
	Fehling's test	+ve	+ve
	Molish test	+ve	+ve
3	**Gums/Mucilage**		
	Water	+ve	+ve
	Alcohol	+ve	+ve
4	**Tannins**		
	Aq. FeCl3 Test	+ve	+ve
	Alc. FeCl3 Test	+ve	+ve
5	**Flavonoids**		
	Lead acetate test	+ve	+ve
	Shinoda test	+ve	+ve
	Mg/Hcl	+ve	+ve
6	**Saponins**		
	Foam Test	+ve	+ve
	Lead acetate test	+ve	+ve
7	**Sterols**		
	Salowaski test	+ve	+ve
	Libberman Burchad	+ve	+ve

Analgesic activity

Among the many methods used for screening of analgesic activity, one of the most commonly employed techniques is Eddy's hot plate method. In this method heat is used as a source of pain. Animals are placed individually on the hot plate maintained at constant temperature ($55^0C \pm 1^0C$). The reaction time i.e. time taken by the animal to lick its hind paw or to leap out planning it on the hot plate it taken as the reaction of painful stimuli. Analgesics increase the reaction time.

Oral administration of the aqueous and ethanol extracts of *Argyreia Nervosa* aerial parts, have shown significant analgesic activity at a dose of 400mg/kg.

The observed analgesic activity was comparable with that effect produced by standard drug pentazocine administration.

Phytochemical analysis of *Argyreia Nervosa* aerial parts extracts had shown the presence of chemical constituents like alkaloids flavonoids, saponins, tannins and sterols. Hence in the present study, it can be assumed that analgesic activity of *Argyreia Nervosa* extracts might be produced due to the presence of flavonoids and sterols.

Table 2: Analgesic activity of aqueous and ethanolic extracts of *Argyreia nervosa* aerial parts extracts.

Group	Treatment	Dose mg/kg	Basal reaction time (sec)				
			0	30	60	90	120
1	Control	-	3.5±0.22	3.5±0.22	4.0±3.6	3.5±0.21	4.16±0.30
2	Standard	10	5.1±0.30	6.66±0.33	9.0±0.42	9.2±0.21	9.22±0.42
3	AEAN	200	3.16±0.30	3.33±0.21	4.16±0.30	4.66±0.22	4.66±0.21
4	EEAN	200	3.60±0.16	3.3±0.22	4.3±0.02	5.33±0.33	5±0.36
5	AEAN	400	4.66±0.33	5.53±0.3	7.8±0.36	7.66±0.33	7.5±0.33
6	EEAN	400	5.10±0.30	5.43±0.30	8.12±0.33	7.3±0.67	7.4±0.21

Standard drug used: Pentazocine
N = 6, significant at p< 0.05, 0.01 and 0.001, ns = non-significant
AEAN = Alcoholic extract of *Argyreia Nervosa*
EEAN = Ethanolic extract of *Argyreia Nervosa*

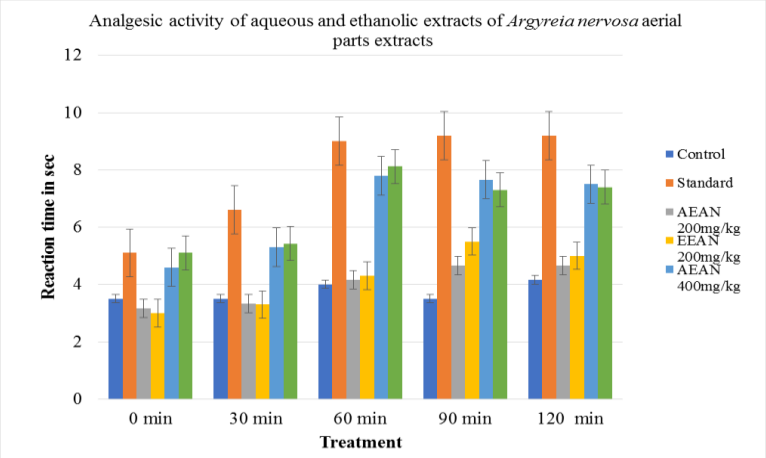

Fig. 1: Graphical representation of analgesic activity of aqueous and ethanolic extracts of *Argyreia Nervosa* aerial parts extracts.

Anti-inflammatory activity

Histamine induced paw oedema is an in vivo model of inflammation; it was selected to assess the anti-inflammatory activity of natural products particularly in the acute phase of inflammation. Oedema formation is due to histamine in the rat is a biphasic event. The first phase is due to the serotonin release, then peak effect is observed at 180 min due to release of kinin like substances and the second phase is caused by the release of protease, prostaglandins and lysosome. Therefore, it can be assumed that the inhibitory effect of all the extracts and diclofenac sodium on histamine induced inflammation could be due to inhibition of the enzyme carbooxygenase, leading to inhibition of prostaglandin synthesis oral administration of the aqueous and ethanol extracts of aerial parts of *Argyreia Nervosa* suppressed the edematous response after 2h and this effect continued up to 4hrs. the observed effect was comparable with the effect that produced by diclofenac sodium administration. Hence in the present study, it can be assumed that anti-inflammatory activity of *Argyreia Nervosa* extracts might be produced due to the presence of flavonoids and sterols.

Table 3: Anti-inflammatory activity of aqueous and ethanolic extract of *Argyreia nervosa*.

Group	Treatment	Dose mg/kg	Paw oedema volume								
			Zero hours	After 1st hour		After 2nd hour		After 3rd hour		After 4th hour	
			Normal paw volume	Mean ± SEM	% ROV	Mean ± SEM	% ROV	Mean ± SEM	% ROV	Mean ± SEM	% ROV
1	Control	-	0.95	0.183±0.401	-	0.51±0.061	-	0.58±0.048	-	0.55±0.072	-
2	Standard	50mg/kg	0.89	0.1±0.0	45.44	0.16±0.021	68.62	0.11±0.031	81.03	0.1±0.034	81.81
3	AEAN	200mg/kg	0.81	0.38±0.098	15.55	0.45±0.921	18.18	0.45±0.013	22.41	0.48±0.095	12.72
4	EEAN	200mg/kg	0.80	0.26±0.021	8.74	0.33±0.042	35.29	0.31±0.031	38.49	0.30±0.076	39.5
5	AEAN	400mg/kg	0.95	0.183±0.016	0	0.36±0.041	29.41	0.33±0.056	43.10	0.21±0.048	61.81
6	EEAN	400mg/kg	0.77	0.24±0.005	36.6	0.21±0.040	58.8	0.15±0.056	61.1	0.31±0.047	43.63

AEAN = Alcoholic extract of *Argyreia Nervosa*
EEAN = Ethanolic extract of *Argyreia Nervosa*

Standard drug used: Diclofenac sodium.
N = 6, significant at P < 0.05, 0.01 and 0.001
ns = non-significant

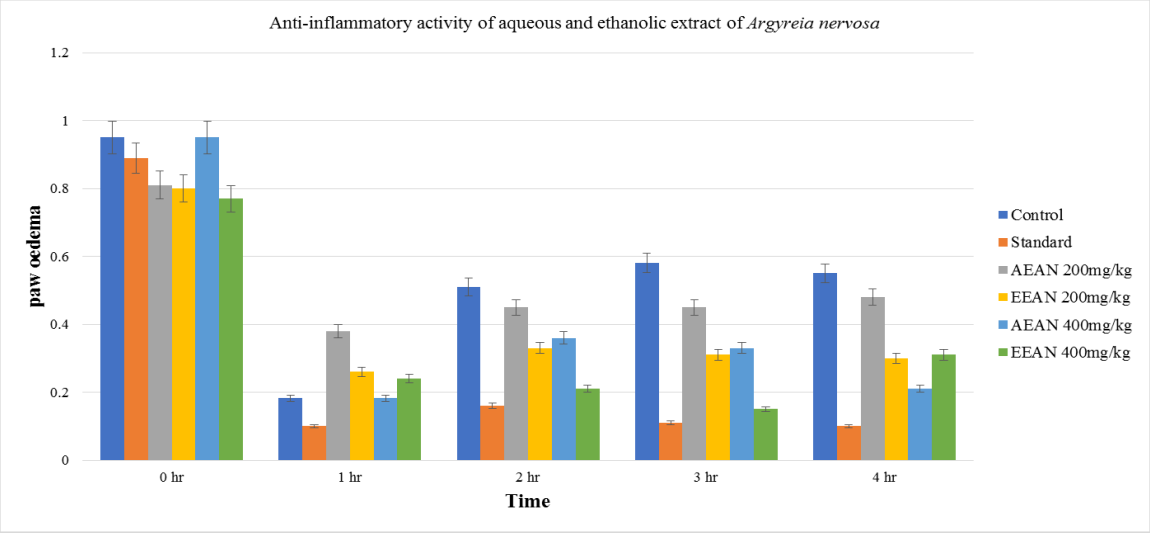

Fig. 2: Graphical representation of paw oedema of anti-inflammatory activity of aqueous and ethanolic extracts of *Argyreia Nervosa* aerial parts extracts.

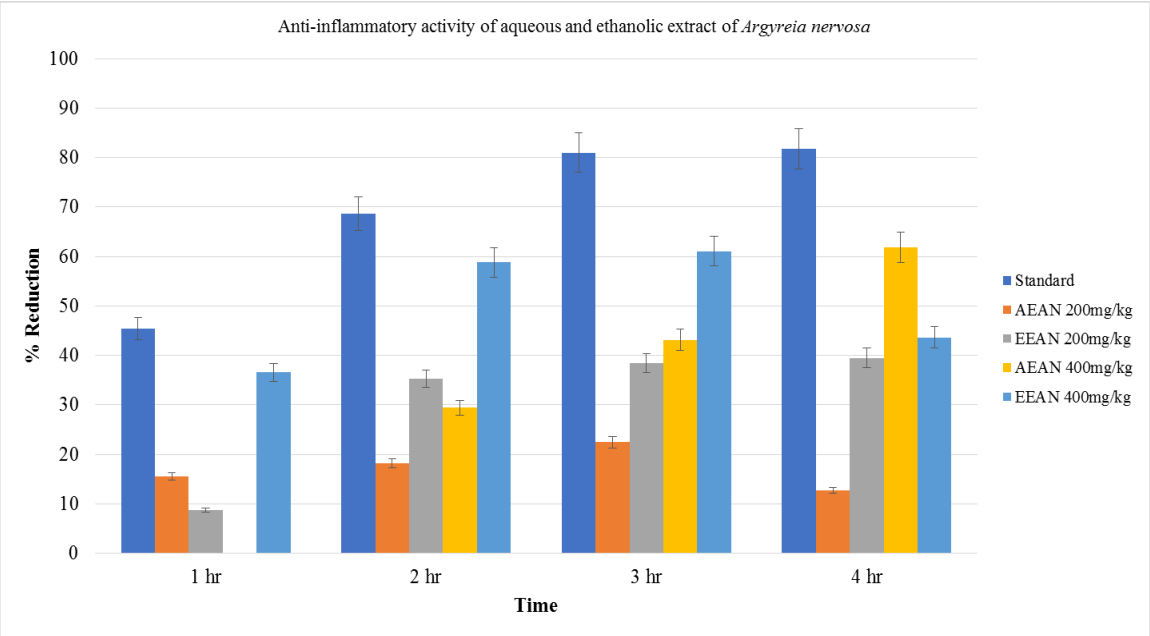

Fig. 3: Graphical representation of anti-inflammatory activity (% reduction of oedema) of aqueous and ethanolic extracts of *Argyreia Nervosa* aerial parts extracts.

CONCLUSION

The present study was carried out to determine the effect of *Argyreia Nervosa* aerial parts extracts an experimental model of analgesic and anti-inflammatory activity.

The *Argyreia Nervosa* aerial parts extracts produced significant analgesic and anti-inflammatory was comparable to that produced by pentazocine, diclofenac sodium, and phytoconstituents flavonoids saponins tannins and sterols in the extracts might be responsible for these activities.

However further studies are required to isolate and identify the active constituents responsible for the activity and also focus on the mechanism of analgesic and anti-inflammatory activity.

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