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IN VIVO ANALGESIC ACTIVITY OF WHOLE AERIAL PART-*ARGYREIA NERVOSA*

K. Jeet*, R. Thakur, S. Choudhary, A. Shukla , A.K. Sharma

Department of Pharmacy, The Pharmaceutical College, Barpali, Orissa, India.

ABSTRACT

Argyreia nervosa is a potent medicinal plant in the Indian systems of medicine. Traditionally it is used as an antibacterial, antifungal, antipyretic, analgesic etc. In the present study the ethyl acetate extract and methanol extract of the whole aerial part from *Argyreia nervosa* was studied for its analgesic activity. Study was carried out on healthy albino mice weighing about 30-40g and healthy wistar strain albino rats weighing about 150-200 g, using acetic acid induced writhing and tail immersion method. It was observed that the ethyl acetate extract and methanol extract produced significant analgesic activity.

Key words: *Argyreia nervosa*, *Argyreia speciosa*, Tail immersion method, Acetic acid induced writhing, Analgesic activity.

INTRODUCTION

The use of plants as medicine is as old as human civilization. People of all ages in both developing and developed countries use plants in an attempt to cure various diseases and to get relief from physical sufferings. Natural products are a source for bioactive compounds and have potential for developing some novel therapeutic agents.

Argyreia nervosa is a Vine Forb/herb (USDA, 2011) belongs to family *Convolvulaceae* in hindi it is known as samundar-ka-pat (Anonymous, 1995). Its botanical synonym (USDA, 2011) is *Argyreia speciosa*. It is distributed throughout India, up to an altitude of 300 m, often cultivated native in India from Assam and Bengal to Karnataka (Anonymous, 1985; Guhabakshi *et al*., 1999; Nadkarni, 1976). Leaves are 7.5-3.0 by 6.3-2.5 cm. (sometimes even larger), ovate, acute glabrous above, persistently white-tomentose beneath, base cordate; petioles 5-15 cm. long, white-tomentose: characteristic odour and slightly bitter taste (Anonymous, 1985; Kirtikar and Basu, 1981). Stem stout, white tomentose, characteristic odour and slightly bitter taste (Kirtikar and Basu, 1981). Traditionally it was used in gleet, gonorrhoea, strangury and chronic ulcers. A preparation 'Fortege' made from this plant along with several other ingredients is used for curing sexual disorders in males. Another drug 'Speman' consisting of several ingredients of plant material including this species, is reported to exhibit anabolic-cum androgen-like activity in mice (Anonymous, 1995), In stomach complaints, sores on foot, small pox, syphilis, dysentery and diarrhoea (Guhabakshi *et al*., 1999).

Corresponding Author

K. Jeet
Email: express_pharma@yahoo.com

MATERIALS AND METHODS

Collection, Authentication and Preparation of Plant Material

The fresh aerial part collected from local area of Barpali, (Dist-Bargarh, Orissa). The plant was authenticated by Botanical Survey of India (BSI), Central National Herbarium Howrah, Kolkata, India. Ref. no. CNN/I-I/49/2010/Tech.II/285. The whole aerial part was dried dried under shade and were powdered by the help of mechanical process. Powder of whole aerial part was stored in a suitable place.

Extraction

The dried powder plant material was extracted with ethyl acetate and methanol, by successive cold maceration method with increasing order of their polarity.

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The powdered drug was extracted for 7 days with each solvent. The extract was then filtered using filter paper and the filtrate so obtained was evaporated in a distillation unit (Harborne, 1998).

Phytochemical Study

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, proteins, saponins and glycosides were carried out on extracts using standard procedure (Shah and Nayak, 2008; Kokate *et al*., 2002).

Animals

Healthy albino mice weighing about 30-40g and Wistar strain albino rats weighing about 150-200g were taken. They were divided into 4 groups having 6 in each, numbered and placed into individual restraining cages. The animals are then allowed to adapt in the cages for 30 minutes before testing. The experiment protocols were approved by the Institutional Animal Ethics Committee prior to the conduct of the animal experiments.

Acute Toxicity Studies

The acute oral toxicity test of the extract was carried out as per revised OECD (Organisation for Economic Cooperation and Development) guidelines 423. The ethyl acetate and methanolic extract of whole aerial part from *Argyreia nervosa* was administered orally to overnight fasted animals at the dose of 250 mg/kg, 500 mg/kg, 1000 mg/kg and 3000 mg/kg of body weight. After administration of the extracts, the animals were observed continuously for the first two hours, for any toxic manifestation. Thereafter, observations were made at regular intervals for 48 hours. Further the animals were under investigation up to a period of 2 week for mortality and general behavior (Kumar and Alagawadi, 2010).

Analgesic Activity

Acetic Acid-Induced Writhing

In this method, Healthy albino mice weighing about 30-40g were pretreated with drugs 45 minutes before induction of writhing. The animals received the standard drug Diclofenac sodium (40 mg/kg, p.o.) which served as reference standard. Analgesic activity of ethyl acetate and methanolic extract of *Argyreia nervosa* 300 mg/kg, p.o. was assessed by counting the number of writhes induced by 0.6% acetic acid (10 ml/kg i.p.) (Koster *et al*., 1959; Turner, 1971). The number of writhes per animal was counted for the next 20 minutes. Percentage protection against abdominal constriction was taken as an index of analgesia.

It was calculated as:

$$\frac{\text{Number of writhing in control group} - \text{Number of writhing in treated group}}{\text{Number of writhing in control group}} \times 100$$

Tail Immersion Method

In this method, Wistar strain albino rats weighing about 150-200g were pretreated with drugs 60 minutes before tail immersion. The animals received the standard drug Diclofenac sodium (45 mg/kg, p.o) which served as reference standard. The distal 2-3 cm portion of mouse-tail was immersed in hot water maintained at 55 ±10C. The time taken by the rats to withdraw the tail from hot water was noted as reaction time. The cut off time was considered 10-12 sec (Turner, 1971).

Statistical Analysis

All results are expressed as mean ± standard error mean (S.E.M). The data was analyzed statistically using ANOVA followed by Dunnett's Multiple Comparison Test using SPSS 10.0 statistical software. The level of significance was fixed at 5%.

RESULTS AND DISCUSSION

Extracts

The dried powder of whole aerial part of *Argyreia nervosa* was extracted with ethyl acetate, methanol by successive cold maceration method. The ethyl acetate and methanol extracts so obtained having yield 3.57% w/w and 4.93% w/w respectively and a general study reveal yield, consistency and color of extracts given in (Table 1).

Table 1. Yield, color and consistency of Extracts

Extracts	%age Yield (w/w)	Consistency	Color	Color under UV
Ethyl acetate	3.57%	Sticky	Greenish black	Brown
Methanol	4.93%	Greasy	Dark black	Dark brown

Preliminary Phytochemical Studies

Ethyl acetate extract of whole aerial part from *Argyreia nervosa* shows the presence of fixed oil, fats, phytosterols, glycosides, flavonoids, alkaloids, tannins and phenolic compounds while methanol extract shows the presence of carbohydrates, protein, amino acids, fixed oil, fats, phytosterols, glycosides, flavonoids, alkaloids, tannins and phenolic compounds.

Acute Toxicity Studies

Acute toxicity studies were carried out to evaluate the drug's toxicity and to determine the minimum effective dose of the drug extracts, using albino rats. No death was observed till the end of the study. The extract was found to be safe up to the dose of 3000 mg/kg hence 1/10th of the tested dose, 300 mg/kg dose was chosen as the experimental dose (Table 2).

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Table 2. Acute toxicity studies

S. No.	Dose (mg/kg)	Observation
1	250	No Death
2	500	No Death
3	1000	No Death
4	3000	No Death

Analgesic Activity

Acetic acid-induced writhing

The ethyl acetate and methanolic extracts of *Argyreia nervosa* 300 mg/kg, p.o. significantly ($P < 0.01$) reduced the number of writhing induced by acetic acid compared to control group. Inhibition of writhing response by ethyl acetate extract of (300 mg/kg) was 66.96% and inhibition of writhing response by methanol extract (300 mg/kg) was 58.65 which is comparable to Diclofenac sodium (Table 3).

Tail immersion method

The ethyl acetate and methanol extract of whole aerial part of *Argyreia nervosa* at doses (300 mg/kg, p.o.) showed significant ($P < 0.05$) increase in latency to tail withdrawal compared to control group. The nociception inhibition of stimulus by ethyl acetate and methnol extracts of whole aerial parts of *Argyreia nervosa* (300 mg/kg) was comparable to Diclofenac sodium (40 mg/kg, p.o) results are given in Table 4.

Table 3. Analgesic activity of whole aerial part of *Argyreia nervosa* by using Acetic acid induced writhing Method

Groups	Treatment	Dose	writhing	% inhibition
Group-I	Control (2% gum acacia)	2ml/kg ,p.o	44.50± 1.28	--
Group-II	Diclofenac Sodium	40mg/kg, p.o	10.20± 1.031**	77.07
Group-III	Ethyl Acetate Extract	300 mg/kg, p.o	14.70 ± 1.50**	66.96
Group-IV	Methanolic Extract	300 mg/kg, p.o	18.40 ± 1.35**	58.65

Each value is Mean ± S.E.M (n=6), **Denotes significant difference when compared to control values at p<0.01

Figure 1. Analgesic activity using Acetic acid induced writhing

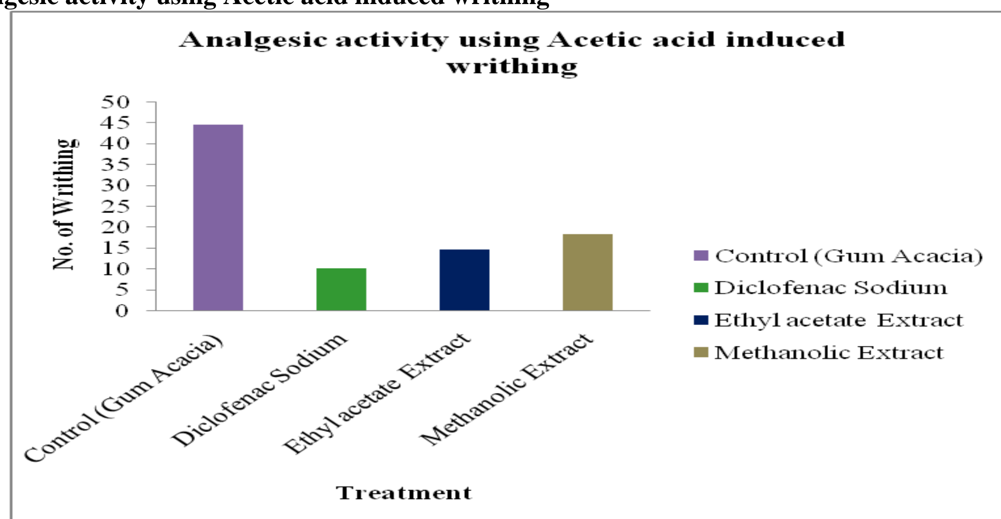
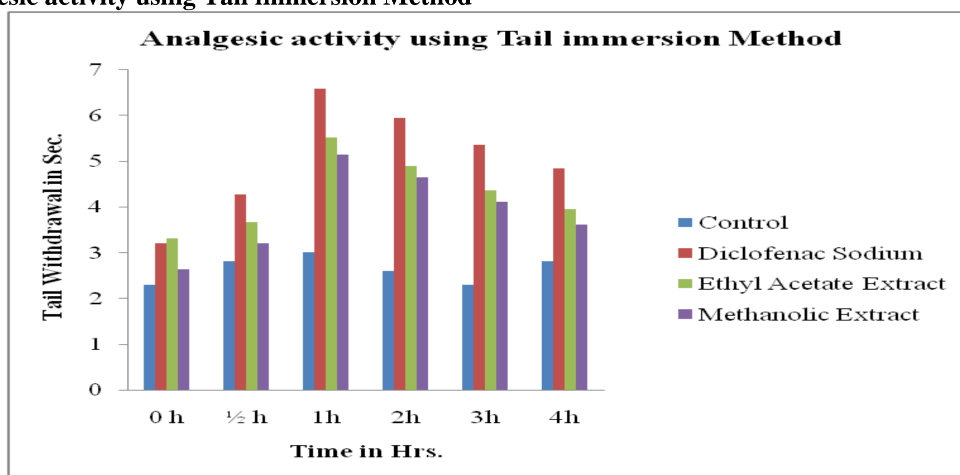


Table 4. Analgesic activity of whole aerial part of *Argyreia nervosa* by using Tail immersion Method

Drug/ Extract	Dose	Reaction time (Second) ± SEM					
		0 h	½ h	1h	2h	3h	4h
Control	2% gum acacia solution, p.o	2.3 ± 0.14	2.8 ± 0.97	3.0 ± 0.2	2.6 ± 0.08	2.3 ± 0.36	2.8± 0.09
Diclofenac Sodium	45mg/kg, p.o	3.2 ± 0.31	4.27± 0.36*	6.58± 0.38*	5.93± 0.47*	5.35± 0.22*	4.84± 0.6*
Ethyl Acetate Extract	300 mg/kg, p.o	3.31 ± 0.8	3.66± 0.15*	5.51± 0.73*	4.89± 0.27*	4.35± 0.52*	3.94± 0.69*
Methanolic Extract	300 mg/kg, p.o	2.63± 0.58	3.2± 0.73*	5.14± 0.56*	4.63± 0.37*	4.1± 0.58*	3.61± 0.47*

Each value is Mean ± S.E.M (n=6), *Denotes significant difference when compared to control values at p<0.05

Figure 2. Analgesic activity using Tail immersion Method



DISCUSSION

Phytochemical investigation ethyl acetate extract shows the presence of fixed oil, fats, phytosterols, glycosides, flavonoids, alkaloids, tannins and phenolic compounds while methanol extract shows the presence of carbohydrates, protein, amino acids, fixed oil, fats, phytosterols, glycosides, flavonoids, alkaloids, tannins and phenolic compounds.

Thus, the analgesic activity of *Argyreia nervosa* could be due to flavonoids and triterpene components (Gokhale *et al*., 2002). Both extracts does not show any toxicity and behavioral changes in mice and rats up to 3000 mg/kg hence doses of 300 mg/kg, p.o. were selected for the present study.

The analgesic effects in various models of pain and inflammation were found to be analogous. The stimulus may be thermal (tail flick, tail immersion, and hot plate tests), mechanical (tail or paw pressure tests), electrical (stimulation of paw, tail or dental pulp) or chemical (writhing and formalin tests) (George *et al*., 2009). Acetic acid-induced abdominal constriction is a sensitive method for screening peripheral analgesic effect of compounds. It causes an increase in concentration of PGE2 and PGF2α in the peritoneal fluid (Bentley *et al*., 1983; Collier *et al*., 1968).

The hot plate method and tail immersion method have been found to be suitable for evaluation of centrally acting analgesics (Woolfe *et al*., 1994). The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PGs may be minimized in this model. In centrally acting analgesic methods, the drug at 300 mg/kg doses was found to be significantly effective in analgesic model for evaluating centrally acting drugs as well as for evaluate peripherally acting analgesics in the acetic acid-induced abdominal constriction method, pain is generated indirectly via endogenous mediators like prostaglandin, whichstimulates peripheral nociceptive neurons. These neuronal fibers are sensitive to both narcotics and non steroidal anti-inflammatory drugs (Collier *et al*., 1968). The both extracts *inhibited* the acetic acid-induced pain increase in latency to tail withdrawal with potency compared to the Diclofenac sodium.

CONCLUSION

The ethyl acetate and methanol extract from *Argyreia nervosa* exhibited analgesic activity in experimental animal models. The results of this study provide a scientific basis for the utilization of *Argyreia nervosa* in traditional medicine. Further studies and tests are needed to explore the exact active principle responsible for the analgesic activity.

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REFERENCES

Anonymous, Flora of Orissa. Orissa forest development co. ltd Bhubaneswar, Orissa, 1995.

Anonymous, Wealth of India. A dictionary of Indian Raw materials and industrial products, CSIR. New Delhi, 1985.

Bentley GA, Newton SH, Starr J. Studies on the anti-nociceptive action of drugs and their interaction with opioid mechanism against. *Br J Pharmacol*. 79, 1983, 125-134.

Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in mouse. *Br Pharmacol*. 32, 1968, 295-310.

George KA, Eric W, David DO, George AK. Antinociceptive Effects of *Newboulia laveis (P.Beauv)* Stem Bark Extract in a Rat Model. *Phcog Mag*. 17, 2009, 49-54.

Gokhale AB, Damre AS, Kulkarni KR, Saraf MN. Preliminary evaluation of anti-inflammatory and anti-arthritic activity of *S. lappa, A. speciosa* and *A. aspera*. *Phytomedicine*. 9, 2002, 433-437.

Guhabakshi DN, Sensarma P, Pal DC. A lexicon of medicinal plant in India. New Delhi, 1.1999: 180-181.

Harborne JB. Phytochemical Methods-A Guide to Modern Techniques of Plant Analysis. 3rd ed., Springer, New Delhi, 1998: 40-227.

Kirtikar KR, Basu BD. Indian medicinal plants, Edn 2, Vol. 3. Lalit Mohan Basu publication, 1981: 1707-1708.

Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Edn 20, Nirali Prakashan, Pune, India, 2002: 108-109.

Koster R, Anderson M, De Beer EJ. Acetic acid for analgesic screening. *Fed Proc*. 18,1959, 412-420.

Kumar S and Alagawadi KR. Hypoglycemic effect of *Argyreia nervosa* root extract in normal and streptozotocin-diabetic rats. *Der Pharmacia Lettre*. 2(2), 2010, 333-337.

Nadkarni KM. Indian Materia Medica, Vol.1, Popular Prakashan, Bombay, 1976: 136-137.

Shah BN, Nayak BS. Experimental Pharmacognosy, Edn 1, Jalandhar, India, 2008: 190-200.

Turner RA.Screening methods in pharmacology. New York: Academic Press, 1971: 100-113.

USDA, Natural resources conservation service website http://plants.usda.gov/java/nameSearch? keywordquery=argyreia+ nervosa&mode=sciname&submsu.x=12&submit.y=9.Accessed April 5, 2011.

Woolfe G, MacDonald AD. The evaluation of the analgesic action of Pethidine Hydrochloride. *J Pharmacol Exp Ther*. 80, 1994,300.