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Identify and Evaluate the Properties of Bark and Leaves of, Saraca Asoca (Roxb.) in Cyclooxygenases Inhibition during Menstruation in Spiny Mouse

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ABSTRACT: Ashoka, Monoon longifolium (Sonn.) possesses various activities such as analgesic, antipyretic, anthelminthic, antidiabetic, fungi toxic, larvicidal activity, anti-microbial activity, CNS (Central Nervous System) depressant activity, antiulcer activity, anti-inflammatory activity etc.

Monoon longifolium leaves was washed, shade dried and grinded to coarse powder. Gum Acacia and Aspirin were used. Daily vaginal smears are taken at noon for five days and examined for oestrous cycle stages.

Monoon longifolium petroleum ether extract showed positive steroid testing. The result demonstrated that standard group showed increase in %reduction in rectal temperature as compared to control group (cP<0.001). Extract 2 showed significant decrease in %reduction in rectal temperature when compared to standard group eP <0.01.The aim of the present study was to evaluate the activity of Monoon longifolium in the management of menstruation problems keeping in mind the pain irregularity etc. during the menstural cycle.

Keywords: Ashoka, Monoon longifolium, Menstruation

I. INTRODUCTION

Saraca asoca, commonly known as Ashoka tree,its botanical name, Saraca asoca, is derived from the Sanskrit word "Ashoka," which translates to "one that relieves sorrow."Saraca asoca is renowned for its efficacy in managing various gynecological issues, especially those related to the menstrual cycle. It acts as a potent uterine tonic, helping to regulate menstrual flow and alleviate menstrual pain. The presence of flavonoids and tannins in Ashoka bark lends it anti-inflammatory and analgesic properties, making it a natural choice for relieving menstrual discomfort.The phytoestrogens present in Ashoka contribute to hormonal balance, aiding in the regulation of menstrual cycles and fertility.

Menstruation is the monthly shedding of the lining of a woman's uterus (more commonly known as the womb). The synonyms of menstruation are menses, menstrual period, cycle or period. The average age of menarche (first onset of menstruation in girls) in females is 12 years.Girls can begin menstruating as early as 8 years of age or as late as 16 years of age. Women stop menstruating at menopause, which occurs at about the age of 50. Menopause is a condition when a woman stops menstruating and production of eggs.Artavadushti (menstrual disorders) refers to health conditions that affect a woman's normal menstrual cycle. From heavy periods to irregular periods, women can experience many different gynecological issues relating to their monthly cycle.

Causes of menstrual cycle irregularities: Pregnancy or breast-feeding, Eeating disorders extreme weight loss or excessive exercising, polycystic ovarian syndrome (PCOS), Premature Ovarian Failure, Pelvic Inflammatory Disease, Uterine fibroids.

Menstrual irregularities are major problems in adolescents producing psychological stress in parents and children. Studies show that the prevalence of menstrual irregularities among South Indian adolescent population seems to be 11.9%. The common menstrual disorders prevalent among themselves are 2 menorrhagia (17.82%), oligomenorrhoea (16.08%), hypo menorrhea (59.56%), dysmenorrhea (49.13%), and premenstrual tension (46.52%).

Mefenamic acid is a member of the anthranilic acid derivatives class of nonsteroidal anti-inflammatory drugs, and is used to treat mild to moderate pain.Mefenamic acid binds the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthetase.

II. PLANT PROFILE

Monoon longifolium (Sonn.) B.Xueis the most ancient tree of India, generally known as a

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"false Ashokbriksh", botanist known as a Monoon longifolium (Sonn.) B.Xue, Kingdom: Plantae

Clade: Tracheophytes
Clade: Angiosperms
Order: Magnoliales
Family: Annonaceae
Genus: Polyalthia
Species: Polyalthia longifolia
Binomial name: Polyalthia longifolia (Sonn.)

Fig: Ashoka leaves

1.1 Geographical source: The native geographic distribution includes India Andaman & Nicobar Islands, Andhra Pradesh, West Bengal, Assam, Arunachal Pradesh, Bihar, Punjab, Rajasthan, Maharashtra, Manipur, Mizoram, Tamil Nadu, Gujarat, Jharkhand, Karnataka, Uttar Pradesh, Delhi, Goa, Kerala, Madhya Pradesh, Chhattisgarh and Sri Lanka.

1.2 Pharmacological Activity: Anticancer Activity, Antihemorrhagic Activity, Antioxytocic Activity, CNS depressant activity, Antidiabetic activity, Analgesic activity, Larvicidal activity

1.3 Chemical Constituent: Oleic, linoleic, palmitic and stearic acids sitosterol, quercetin, kaempferol, quercetin, apigenin- 7-0-p-D-glucoside, Glycosides, lyoniside, nudiposide, 5-methoxy- 9-β-xylopyranosyl, isolariciresinol, and schizandriside, and three flavonoids, epicatechin, epiafzelechin-(4β→8)-epicatechin and procyanidin B2.

III. MATERIAL AND METHOD

1.4 Collection of Bark and Leave: The leaves of the plant were collected in the month of July 2023 form different parts/district of Chhattisgarh state.

1.5 Identification and Authentication of Collected Plant: Plant parts were identified and authentication from department of botany GuruGhasidas university Bilaspur,Chhattisgarh

1.6 Washing And Shade Drying: Plant samples gathered for preparing undergo a washing process to remove contamination from particles that adhere, such as dust and other contaminants. washing plants dried at room temperature [24±5]. Samples of plant tissue were reduced to the particle size of 0.5 to 1.0 mm to ensure homogeneity and facilitate the destruction of organic matter.

1.7 Extraction Of Monoon longifolium: The Monoon longifolium leaves was washed, shade dried and grinded to coarse powder. Approximately 700 gm of dried powder were Extracted successively with decreasing polarity range such a petroleum ether, ethyl acetate, Methanol, and water at temperature ranges between 40-60 ° C using constant heating Soxhlet apparatus. For 15 cycles, the Extract was continued. The Extract was finally filtered and concentrated to dry weight.

1.8 Isolation and Characterization of Extract: Thin Layer Chromatography is a method used to isolate mixtures that are not volatile. The experiment is performed on an aluminum foil, plastic or glass sheet that is covered with a thin layer of adsorbent material. Aluminum oxide, cellulose, or silica gel is the material normally used.When the separation is complete, each element appears as vertically separated spots. Each spot has a factor of retention (Rf) expressed as:

Rf = Dist. travelled by Sample / Dist. travelled by Solvent

1.9 Procurement of Animals:

- Experimental animals were obtained from Animal House Swiss albino mice (20–25 g) and were acclimatized to the laboratory for 1 week prior to the experiment. All the experimental animals had free access
- **Selection of animals:** Healthy adult spiny female mice weighing around 30g.
- **Vaginal smears:** Daily vaginal smears are taken at noon for five days and examined for oestrous cycle stages.

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- **Selection of animals:** 15-20 rats with regular cycle and in pro-estrous stage are selected for study.
- Menstrual cycle in female spiny mouse is of 4 to 5 days. If pregnancy or pseudo pregnancy is not there the menstrual cycle continues.

1.10 Drugs: Gum Acacia and Aspirin were obtained from Himedia.

IV. PHARMACOLOGICAL STUDY

1.11 Analgesic activity:: The control group (group I) was administered with 5% gum acacia, the standard group (group II) received aspirin (100 mg/kg) and the research groups (group III and IV) was given the research drug at doses of E1 and E2 respectively. The assessment of antipyretic activity was carried out using Brewer's yeast induced pyrexia in spiny mouse by the method as described by Loux et al. Mice were fasted overnight with water ad libitum before the experiment. The normal body temperature of each animal was measured by digital tele-thermometer (IMCORP, Ambala, India) and recorded. Pyrexia was induced by subcutaneously injecting 20% w/v Brewer's yeast (10 mL/kg), suspended in normal saline, into the animal's dorsum region. The control group (group I) was administered 5% gum acacia, the standard group (group II) received aspirin (100 mg/kg) and the research groups (group III and IV) was given the research drug at doses of E1 and E2 respectively. The rectal temperature was recorded.

1.12 Hot Plate Test: The control group (group I) was administered with 5% gum acacia, the standard group (group II) received aspirin (100 mg/kg) and the research groups (group III and IV) was given the research drug at doses of E1 and E2 respectively.

Evaluation of analgesic activity of the Extract was also carried out using hot plate method [2]. The rats were placed on a hot plate maintained at 55℃ within the restrainer. The reaction time (in seconds) or latency period was determined as the time taken for the rats to react to the thermal pain by licking their paws or jumping.The reaction time was recorded before (0 min) and at 15, 30, 45, and 60 min after the administration of the treatments.
The maximum possible analgesia (MPA) was calculated as follows:
MPA = Reaction time for treatment − reaction time for saline 45 sec − reaction time for saline × 100.

V. RESULT

1.13 Percentage Yield of Different Solvent Extracts of Monoon longifolium

Plant Name	Extracts	Color and consistency	% Yield (w/w)
MONOON LONGIFOLIUM	Pet. Ether	Brownish yellow and sticky	1..85%
	Ethyl Acetate	Brown sticky	3.25%
	Methanol	Brown and semisolid	7.65%
	Aqueous	Dark Brown	9.65%

1.14 Extraction Of Monoon longifolium

Fig:Pet-Ether Extract of S.A.

Fig: Ethyl Acetate Extract of S.A.

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Fig: Methanol Extract of S.A.

Fig: Aqueous Extract of S.A.

1.15 Phytochemical Screenings

S. No	Phyto- chemical	Name of Tests	PEML	EAML	MEML	AQML
1.	Alkaloids	Mayer's Test	-	-	+	-
		Wagner's Test	-	-	+	-
		Dragon draft's Test	-	-	+	-
		Hager's Test	-	-	+	-
2.	Glycoside	Modified Brontrager's Test	-	+	+	+
		Legal's Test	-	+	+	+
3.	Tannins	Gelatin Test	-	+	+	+
4.	Phenols	Ferric Chloride Test	-	+	+	+
5.	Flavonoids	Alkaline Test	-	+	+	+
		Lead Acetate Test	-	+	+	+
6.	Saponins	Froth's Test	-	-	-	+
		Foam Test	-	-	-	+
7.	Steroids	Salkowaski Test	+	-	-	-
		Libermann Burchard's Test	+	-	-	-

Note: + Present, -Absent

(PEML: Pet-Ether Extract of S.A., EAML: Ethyl Acetate Extract of S.A., MEML: Methanol Extract of S.A., AQML: Aqueous Extract of S.A.)

1.16 The TLC-Developed Solvent System for the Methanol Extract of Monoon longifolium

Extract no	Extract no
Rf Values	Rf Values
Extract 1	Extract 1
0.210	0.210
Extract 2	Extract 2
0.387	0.387

1.17 Isolation of Extract by Column Chromatography

S. No	Wavelength (nm)	Absorbance
1.	668	0.6339
2.	610	0.1226
3.	538	0.1502
4.	508	0.1536
5.	414	1.5349
6.	278	0.4824

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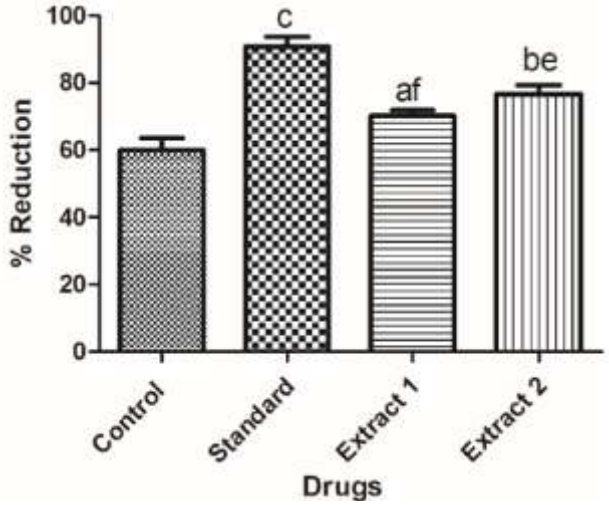
1.18 Analgesic activity

The result demonstrated that standard group showed increase in %reduction in rectal temperature as compared to control group (cP<0.001). Further Extract 2 showed significant decrease in%reduction in rectal temperature when compared to standard group eP <0.01. The result demonstrated that Extract 1 showed best promising effect in reducing the rectal temperature as compared to standard group (fP<0.001).



Fig;Effect of drugs on Antipyretic activity. Data were analyzed by one way ANOVA followed by the Newman-Keuls multiple comparison test. Values are expressed as mean + SEM, where aP <0.05, bP <0.01, cP<0.001 as compared to control. dP <0.05, eP <0.01, fP<0.001 as compared to standard.

1.19 Hot Plate Test:

The results demonstrated that significant increase in reaction time is noted in standard groupwhencompared to control group in 0, 15, 30, 45 and 60 min. Further, extract 1 and Extract 2 showed increase in reaction time as compared to control group in 0, 15, 30, 45 and 60 min. Among them Extract 1 showed more pronounced effect than Extract II.

TREATMENTS	REACTION TIME IN SECONDS				
	0 MIN	**15 MIN**	**30 MIN**	**45 MIN**	**60 MIN**
Control	30.67+4.87	28.57+4.87	24.76+6.37	24.87+6.07	24.98+6.88
Standard	32.89+7.98	27.76+5.65	31.87+7.98	34.76+2.98	31.87+1.46
Extract 1	30.88+4.08	27.67+2.17	25.78+3.97	27.17+3.78	26.62+4.08
Extract 2	30.87+4.71	26.54+3.12	24.76+4.87	26.16+4.32	25.37+3.80

Fig: Effect of drugs on Hot plate. Data were analyzed by one way ANOVA followed by the Bonferoni Post hoc test multiple comparison test. Values are expressed as mean + SEM, where aP <0.05, bP <0.01, cP<0.001 as compared to control. dP <0.05, eP <0.01, fP<0.001 as compared to standard.

VI. DISCUSSION

The reproductive system of a female, unlike men, shows regular cyclic changes that teleologically may be regarded as periodic preparation for pregnancy and fertilization. In primates and humans, the cycle is a menstrual cycle, and its most conspicuous feature is the periodic vaginal bleeding that occurs with the shedding of uterine mucose (menstruation). The length of the cycle is notoriously variable, but an average figure is 28 days from the start of one menstrual period to the start of the next. By common usage, the days of the cycle are identified by number starting with the first day of menstruation. It begins at puberty, ranging from the ages of 10 to 16, and ends at menopause at an average age of 51. Menstruation is the scheduled shedding of the lining of a woman's uterus (more commonly known as the womb). The symptoms of menstruation are menses are cramps, increase in body temperature cycle or period. The menstrual blood, which is partly blood and partly tissue from the inside of the uterus flows from uterus through the cervix and out of the body through the vagina. Menstrual disorders are a common presentation by late adolescence; 75% of girls experience some

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problems associated with menstruation including late, irregular, painful, and heavy menstrual bleeding, which are the principal reasons for the physician office visits by adolescents.

According to the ancient texts known as the "Charaka Samhita" the menstrual cycle should be regular and between twenty-seven and thirty days long. We have selected spiny mouse for our study as it resembles the human body very much. Monoon longifolium the most ancient tree of India, generally known as a "False Ashokbriksh", botanist known as a Monoon longifolium (Sonn.). Further, leaves of Monoon longifolium possess antihemorrhagic property and antianxiolytic activity, that's why the present study demonstrates about the cyclooxygenase inhibition in spiny mouse to reduce the sign and symptoms of menstruation cycle. Arachidonic acid pathway regulates many physiological problems of body like generation of prostaglandins with the help of COX enzyme. So, to inhibit this pathway offers a new therapeutic area to manage menstrual problems

To establish the properties of Monoon longifolium, in the present study we explored the effect of saraca using two models. First is analgesic activity and second is hot plate method. First of all, we will discuss about the analgesic activity of Monoon longifolium. The result demonstrated that standard group showed increase in % reduction in rectal temperature as compared to control group. Extract-2 showed significant decrease in % reduction in rectal temperature. The resultdemonstrated that Extract 1 showed best promising effect in reducing the rectal temperature as compared to standard group. Extract 1 is best to decrees the menstruation cycle problems.

After giving the inference from analgesic activity protocol, to establish the effect of Monoon longifolium, further we have evaluated the effect on hot plate method. For the effective management of menstruation cycle, the arachidonic acid pathway should be suppressed. In hot plate method the activity of drug was assessed. The hot plate test is one of the oldest and most widely used experimental methods to assess nociception in rats and mice. The test consists of placing a rodent on an enclosed hot plate and measuring the latency to lick a hind paw or jump out of the enclosure. The advantages of this test are that it is objective, quantifiable, can be administered repeatedly without causing inflammation, and assesses supraspinal-organized responses to a noxious stimulus. There appears to be a good correspondence between drugs that produce antinociception on the hot plate test and drugs used clinically to treat pain. Similar to the result of analgesic activity, extract 1 showed increase in reaction time as compared to Extract 2, which clearly indicates that Extract 1 is best to combat the menstrual symptoms. Although these are preliminary data on the basis of preclinical trials. Further extensive pharmacological study should be carried out to establish the effect Monoon longifolium on the irregularities of menstruation cycle. Preliminary study suggests best result with Extract 1. However, various drugs are there in the market, but search for a better herbal agent is the need of the hour to reduce the side effect of synthetic drugs.

VII. CONCLUSION

The aim of the present study was to evaluate the activity of Monoon longifolium in the management of menstruation problems keeping in mind the pain irregularity etc. during the menstrual cycle. For this we have Extracted out the active drug that is Extract 1 and II from Monoon longifolium. Pharmacological study proved that efficacy of Extracts of Monoon longifolium. Extract I proved its efficacy over Extract II to reduce the menstrual problems. The herbal drugs for management of these types of disorders are quite important in now a day. The study demonstrated the good effect of Monoon longifolium over menstruation problems by inhibiting arachidonic acid pathway.

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