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Phytochemical evaluation and Analgesic activity of fresh juice of young stem (tender) bark of *Azadirachta indica* A. Juss

Pravin V. Gomase [1]*, Priti S. Shire [2], Sayyed Nazim [3], Amol B. Choudhari [4], Siraj Shaikh [5], Ashish Khairnar [6]

[1, 3- 6] *Ali-Allana College of Pharmacy, Akkalkuwa. Dist- Nandurbar*
[2] *R. C. Patel Institute of Pharmacy, Shirpur. Dist. - Dhule*

ABSTRACT

The aim of present study was to assess the phytochemical evaluation and analgesic activity of fresh juice of young stem (tender) bark of Azadirachta indica A. Juss. The fresh juice of young stem bark of Azadirachta indica was collected and dried under Lyophilizer and fresh extract can be obtained. The fresh juice of young stem bark of Azadirachta indica was studied for its in-vivo analgesic activity by using the Eddy's hot plate method And Heat conduction method response in rats. The time course study was performed to find the peak time for the maximum analgesic activity. The effective dose of the extract for analgesic activity was calculated from dose-response curve by using the Eddy's hot plate method And Heat conduction method response in rats. In both of the cases diclofenac sodium was used as standard drug. In both of Eddy's hot plate method And Heat conduction method response in rats writhing response method; the intraperitoneal administration of fresh juice of young stem (tender) bark of Azadirachta indica A. Juss (200mg/kg, 300 mg/kg and 500 mg/kg) induced a significant analgesic activity in a dose-dependent manner respectively. The plant may have the phytoconstituents which inhibit cyclooxygenase enzyme or act on central opioid receptors.

Keyword: Analgesic activity, *A. indica.* Diclofenac sodium.

INTRODUCTION

There is an increasing demand for the medicinal plants in developing countries like India. Attention need to be given to assess the medicinal value of such plants to explore the potential drugs out of it. Due to having adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid

great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs (Kumara, 2001)[1].

Biswas et al., (2002) have also shown that different types of extracts from various parts of neem tree (bark, seed, leaf) have analgesic, anti-inflammatory, anti-pyretic, immunostimulant, hypoglycaemic, anti-ulcer, anti-fertility, anti-malarial, antibacterial, antifungal, anti-viral, anti-carcinogenic, antioxidant, hepatoprotective effects.

More than 135 compounds have been isolated from different parts of neem. Some of them such as nimbin, nimbinin, nimbidin, nimbolide and nimbidic, are biologically active [2].

Azadirachta indica (Family: Meliaceae) is a fast-growing tree that can reach a height of 15-20 m, rarely to 35-40 m. According to Ayurevedic text it is used for analgesic, anti-inflammatory, anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, anti-infertility, sedative and skin disease[1.] The main active constituents of the plant are nimbin, nimbinin, nimbidin, limocinol, limocinone, azadirol, naheedin, azadironolide, limbocinin[1]. A literature survey reveals that no systematic approach has been made to study the analgesic activity of fresh juice of young stem bark of *A. indica* A. Juss plant. [2, 3, 4]. In the present work, we have investigated Analgesic activity of fresh juice of young stem bark of *A. indica* against diclofinac sodium.

MATERIALS AND METHODS

Plant materials

Young (tender) stem barks of *A.indica* A. Juss tree were collected from Jalgoan Department, India. A qualified botanist of Go-Vigyan Anusandhan Kendra, Nagpur, India, authenticated raw plant material used in the activity.

Physical Evaluation

Ash value

1 gm powdered drug was taken in a tarred silica crucible previously dried and weighed. It was ignited in a furnace until free from carbon. The ash obtained was weighed [5, 6].

Acid insoluble ash

To the crucible containing total ash, 25 ml of dilute hydrochloric acid was added, covered with a watch glass and boiled gently for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water until the filtrate is neutral. It was dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccators for 30 minutes, and then weighed without delay. [5, 6]

Preparation of fresh Juice

The authenticated plant parts i.e. young (tender) stem bark of *Azadirachta indica* A. Juss was collected and scrap by knives. The pieces of young stem bark were weighed and to that measured quantity of water were added and juice was made in mixer. Juice was separated by squeezing the material through clean muslin cloth and filtered; this clear liquid was allowed to dry in Lyophilizer (CAT NO. MSW 137) at reduce pressure for freeze drying. So that it stop the degradation of sensitive constituents, that may be present in the juice, till all the water got evaporate and complete dry powder was formed. The dry juice was transferred to air tight glass or plastic container. This container was placed inside a vacuum container to avoid attack of moisture.

Preliminary Phytochemical Screening of juice extracts (Khandelwal, K., 2000; Hambone, J., 1973)

Phytochemical screening of fresh juice extract of *A. indica* A. Juss family, *Meliacea* for the presence of these secondary metabolite[4]; Alkaloids (Draggendraffts), flavonoides (Shinoda test), saponins (Frothing test), tannins (5 % Ferric chloride), terpenoides (2, 4- dinitro-phenyl hydrazine), carbohydrates (Molish's test) were evaluated according to the methods described by Khandelwal. 2000 [5].

The preliminary phytochemical study reveals the presence of Alkaloids, Glycosides, and tannins.

Thin layer Chromatographic study [7, 8]

Thin layer chromatography (TLC) is mainly use qualitatively for screening of different plant extracts which serve as a very important tool in the overall Phytochemical research studies.

Preparation of TLC plates

The glass plates of different sizes like 20 × 10 cm, 20 × 5 cm and 10 × 5 cm were used for TLC study. The silica gel G was used as a stationary phase and water as a solvent for preparation of slurry.

Pouring technique was used for the preparation of TLC plates. In this method, the slurry was poured on the plates; the plate was then tipped back and forth to spread the slurry uniformly over the surface. The prepared plates were air dried and then activated in the oven at 105^0C for 30 minutes

Application of samples

Standard Borosil glass capillaries were used for applying the samples on the TLC plates. The spot was applied at 1 cm from the end of plate. After application, the spot was allowed to dry.

Preparation of saturated chamber

Ethyl acetate: Methanol (9.5:0.5) was used as mobile phase. The chamber was saturated prior to use so as to avoid unequal solvent evaporation, losses from the developing plate which can lead to various types of random behavior and edge effect.

Development of plate in saturated chamber

The plate was kept in saturated chamber and care was taken that the solvent system level was below that of the spot. The plate was kept until the solvent front ascended approximately 10 cm. The plate was removed from the chamber and air dried. Plate was initially observed under UV radiation and fluorescence was noted. It was then kept in iodine chamber and the Rf values of spots were note.

The plate was air dried once again and sprayed with visualizing reagent i.e. Vanillin sulphuric acid (1.0%)

When Vanillin sulphuric acid (1.0%) was used as a visualizing reagent the plate was kept in oven at 105^oC for 10 minutes for visualization.

Vanillin sulphuric acid reagent

It is fresh solution of 1.0% vanillin in sulphuric acid, used to detect terpens

Pharmacological Study

Test Animal

The experimental protocol was submitted and approved by Institutional Ethical Committee (IAEC No. 648/02/C/CPCSEA), J. L. C. College pharmacy, Nagpur, India. Wister albino rats (150-200 g) of approximate same age were employed in this investigation. The animals were fed with standard pellet diet and water and ad libitum. They were housed under standard conditions of temperature 22^0 C ($\pm$ 3^0 C) humidity 35 % to 60 %, and light (12:12 hr light/dark cycle) in polypropylene mice cage. The animals received the drug treatments by oral gavages tube.

Chemicals

Diclofenac sodium was obtained as a gift sample from German Remedies Ltd., Mumbai for research, and the other chemicals and reagents used were of analytical grade.

Acute toxicity studies

Acute toxicity studies were carried out on Wister albino rats according to method proposed by Ghosh. Fresh juice of young stem bark of A. indica extract at doses of 100, 300, 1000 and 3000 mg/kg body weight were administrated to separate group of rats (n=6), after overnight fasting. Subsequent to administered of drug extracts, the animals were manifestations, like increaser motor activity, salivation, clonic, convulsions, coma and death. Subsequent observation was made at regular interval for 24 hr and the animals were observed for further one week and the extracts were not toxic up to 3000mg/kg body weight.

Analgesic activity [9, 10]

Analgesic activity of fresh juice of young stem (tender) bark of *A.indica* A. Juss extract studied by eddy's hot plate and heat conduction method.

All the experiments were conducted on an isolated and noiseless condition. The analgesic activity was evaluated by the Eddy's hot plate method and by heat conduction method using Analgesiometer in rats. Fresh juice extract of young stem (tender) bark of *Azadirachta indica* A. Juss administered orally. The standard drug Diclofenac sodium was administered in the form of solution in water for injection as vehicle. For the assessment of analgesic activity in each method the animals of either sex were divided into five groups each composed of six animals. All groups received intraperitoneal injection (maximum 1 ml as per ethical norms).

Group I: *Control animals received 5% Tween 80 at the dose of 10 ml/kg. Response) were noted at 0, 30 min, 60 min and 90 min and 120 min. As the reaction time*
Group II: *Animals received standard Diclofenac sodium at the dose 9mg/kg.*
Group III: *Animals received Juice Extract (200 mg/kg)*
Group IV: *Animals received Juice Extract (300 mg/kg)*
Group V: *Animals received Juice Extract (500 mg/kg)*

Heat conduction method

The animals were divided into five groups of 6 animals each. Group I served as control. Group II served as standard and were injected Diclofenac sodium (9 mg/kg) intraperitonially. Group III, Group IV and V were treated orally with fresh juice of extract of *A. indica* of 200 mg/kg, 300 mg/kg and 500 mg/kg body weight respectively. After one hour, the tip of tail was dipped up to 5 cm into hot water maintained at 58°C. The response time was noted as the sudden withdrawal of the tail from the hot water. Cut off time of 10 seconds was maintained to avoid damage to the tail for all groups. The time required for flicking of the tail, was recorded, to assess response to noxious stimulus [9, 10].

Eddy's hot plate method

The animals were divided into five groups of 6 animals each. Group I served as control. Group II served as standard and were injected Diclofenac sodium (9 mg/kg) intraperitonially. Group III, Group IV and Group V were treated orally with fresh juice of young stem bark of *A.indica* A. Juss extract of 200 mg/kg, 300 mg/kg and 500 mg/kg body weight respectively. The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds. [9, 10]

Statistical analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test. Comparison between control and drug treated groups were considered to be significant. All values are expressed as mean ± SEM.

RESULT

The preliminary phytochemical study reveals the presence of Alkaloids, Glycosides, and tannins.

Table 1: Data showing Physical evaluation of young stem bark of *A. indica* A.Juss.

Sr. No.	Physical Evaluation	Results
1	Total Ash Content	6.0 ± 2.0 %
2	Acid Insoluble Ash	0.2 ± 0.7 %.

Figure 1- Shows Chromatogram of young stem bark juice of *Azadirachta indica* A. Juss.

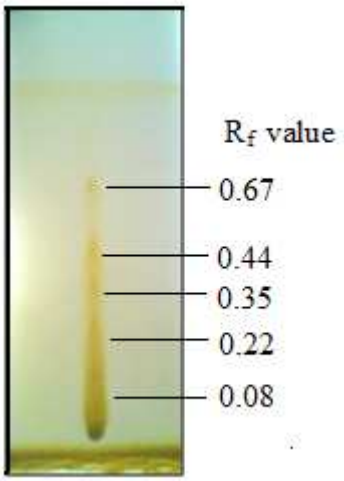


Table 2 : Shows Thin layer chromatography of fresh juice of young stem bark of *Azadirachta indica* A. Juss

Extract	Solvent System	No. of spot			Rf Value
		UV light	Iodine Vapour	Vanillin Sulphuric Acid	
		2 spot observed	5 spot observed	5 spot observed	
Aqueous extract of *Azadirachta indica* A. Juss	Ethylacetate : Methanol (9.5 : 0.5)	Sky blue Faint blue	Yellow Yellow Yellow Yellow Yellow	Light Brown Brown Light pink Pink Brown	0.67 0.44 0.35 0.22 0.08

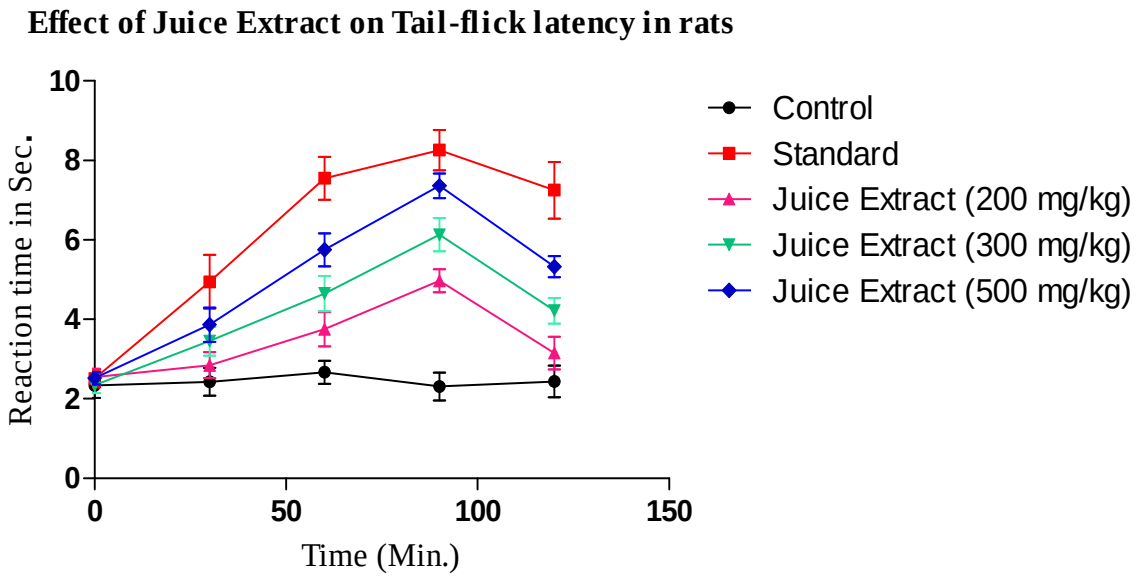
Heat conduction method : Animals treated with 200, 300, 500mg/kg body weight fresh juice extract of *Azadirachta indica* showed significant increase in the tail flick latency compared to control. The tail flick latency at a dose of 500mg/kg body weight of Azadirachta indica (maximum dose) was found to be 7.36 sec after 90 min of drug treatment whereas the standard drug diclofenac sodium showed the tail flick latency 8.26 sec (Table-3) (Figure-2). The activity was also found to be a significant activity.

Table 3: Shows analgesic activity of fresh juice of young stem (tender) bark of *Azadirachta indica* A. Juss. By heat conduction method

Treatment	Tail- flick latency in Sec (Mean ± S.E.M.) at time (min)				
	0 min	30 min	60 min	90 min	120 min
Control	2.34 ± 0.32	2.43± 0.35	2.67 ± 0.29	2.31 ± 0.35	2.44 ± 0.40
Standard	2.51 ± 0.22	4.95 ± 0.68**	7.55 ± 0.54**	8.26 ± 0.51**	7.25 ± 0.71**
Juice Extract (200 mg/kg)	2.55 ± 0.20	2.85 ± 0.33	3.75 ± 0.43*	4.97 ± 0.29**	3.15 ± 0.41
Juice Extract (300 mg/kg)	2.35 ± 0.20	3.45 ± 0.36**	4.65 ± 0.45**	6.13 ± 0 .42**	4.22 ± 0.32**
Juice Extract (500 mg/kg)	2.52 ± 0.16	3.87 ± 0.43**	5.75 ± 0.41**	7.36 ± 0.31**	5.33 ± 0.27**

*All values are expressed as mean ±S.E.M. (n= 6); ** P< 0.01, * P < 0.05 significant compared to control*

Figure 2- Effect of fresh juice extract *Azadirachta indica* A. Juss on Tail- flick latency in rats



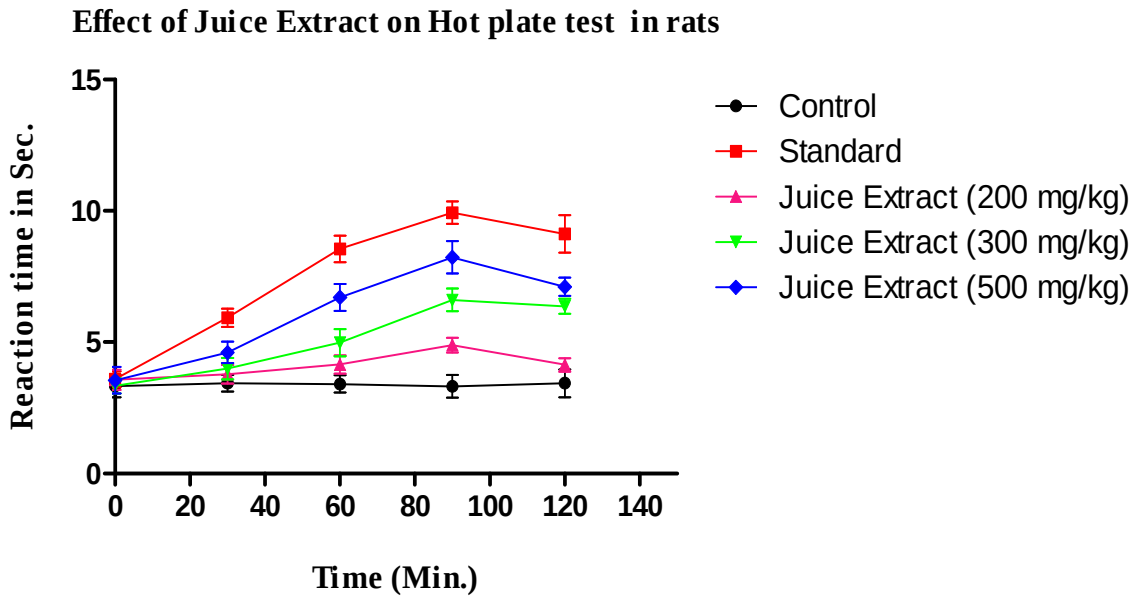
Hot plate method: Animals treated with 200, 300, 500mg/kg body weight fresh juice extract of *Azadirachta indica* showed significant and dose dependent analgesic activity in thermal stimulated pain (hot plate test) in rats. The reaction time at a dose of 500mg/kg body weight *Azadirachta indica* (maximum dose) was found to be 8.23 sec after 90 min of drug treatment whereas the standard drug diclofenac sodium showed the tail flick latency 9.93 sec. (Table-4) (Figure-3).

Table 4: Shows analgesic activity of fresh juice of young stem (tender) bark *of Azadirachta indica* A. Juss. By Eddy's hot plate method

Treatment	Reaction time in Sec (Mean ± S.E.M.) at time (min)				
	0 min	30 min	60 min	90 min	120 min
Control	3.33 ± 0.42	3.44± 0.32	3.41 ± 0.33	3.32 ± 0.43	3.44 ± 0.53
Standard	3.59 ± 0.32	5.93 ± 0.36**	8.55 ± 0.51**	9.93 ± 0.42**	9.12 ± 0.71**
Juice Extract (200 mg/kg)	3.57 ± 0.39	3.78 ± 0.35	4.15 ± 0.34*	4.89 ± 0.28**	4.14 ± 0.28
Juice Extract (300 mg/kg)	3.34 ± 0.29	3.99 ± 0.42**	4.98 ± 0.51**	6.61 ± 0 .42**	6.37 ± 0.29**
Juice Extract (500 mg/kg)	3.55 ± 0.51	4.61 ± 0.42**	6.71 ± 0.52**	8.23 ± 0.63**	7.11 ± 0.32**

*All values are expressed as mean ±S.E.M. (n= 6); ** P< 0.01, * P < 0.05 significant compared to control*

Figures 3 - Effect of fresh juice extract *Azadirachta indica* A. Juss on Hot plate test method in rats



DISCUSSION

Young (tender) stem barks of *A.indica* A. Juss, family *Meliacea*, were collected and authenticated raw plant material used in the activity.

The authenticated and collected plant parts i.e. young (tender) stem bark of *Azadirachta indica* A. Juss were scrap by knives and juice was made in mixer. Juice was separated by squeezing and allowed to dry in Lyophilizer (CAT NO. MSW 137) at reduce pressure for freeze drying. So that it stop the degradation of sensitive constituents, that may be present in the juice, till all the water got evaporate and complete dry powder was formed. The dry juice was transferred to air tight glass or plastic container. This container was placed inside a vacuum container to avoid attack of moisture. Preliminary phytochemical screening were perform, it was found that the dried fresh juice of young stem bark of *A. indica* A.Juss contains carbohydrates, proteins containing sulphur, saponins, flavonoids, terpenoides, tannins & phenolics [5]. Thin layer chromatography (TLC) was carried out mainly for use qualitatively for screening of different plant extracts which serve as a very important tool in the overall Phytochemical research studies.

For determination of dose, acute toxicity study was carried out in which no mortality was found; hence the doses of 200 mg/kg, 300 mg/kg, and 500mg/kg body weight were selected approximately for pharmacological studies.

A. indica Juice extract possesses potent analgesic activity with the help of Eddy's hot plate method And Heat conduction method response in rats at a dose 500 mg/ kg, which was comparable to that of standard Diclofinac sodium.

CONCLUSION

In conclusion, we can confirm that the fresh juice of young stem bark of *A. indica* A. Juss extracts showed analgesic properties. However, further study is needed in order to understand the precise mechanism. In future experiments, studies with purified fractions of the extract can be conducted for further pharmacological and toxicological characterization, such as the research of the mechanisms involved in the central and peripheral analgesic effect.

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