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An evaluation of analgesic activity of leaf and stem bark extracts of ficus religiosa in wistar rats

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***Abstract*---**Introduction: Pain is an unpleasant sensory and emotional experience associated with actual or potential damage. Non- steroidal anti-inflammatory drug (NSAIDs) & Opioids are wide analgesics, but because of adverse effects, their use is limited. Ficus Religiosa is a traditional medicinal plant, its various parts have been used in treating some conditions. Aims & Objective: To evaluate the analgesic activity of methanolic extract of leaves and stem bark of Ficus Religiosa at different doses. Materials & Methodology: This study was conducted in the Department of Pharmacology. Two doses of methanolic extract of leaves (100 & 200 mg/kg) and stem bark (125 & 250 mg/kg) of ficus religiosa were used. Wistar rats were used to

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evaluate analgesic activity and it was evaluated by using analgesiometer by tail flick method. Phytochemical screening of both the extracts were also done. Results: Both the extracts at their respective doses showed significant analgesic activity as compared with the control group, whereas it was not comparable with the standard drug ibuprofen (40 mg/kg p.o). Conclusion: Therefore, we conclude that both the extracts of ficus religiosa were showing analgesic activity. But this analgesic activity is not comparable with the standard drug Ibuprofen.

***Keywords*---**Ficus Religiosa, Methanolic extract, Tail flick, Analgesiometer, Phytochemicals.

Introduction

The International Association for the Study of Pain (IASP) has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" [1] The attempt to understand pain represents one of the oldest challenges in the history of medicine. Pain has a valuable role in medical action, as the symptom par excellence and, therefore, as a precious and meaningful tool.
Pain is the usual experience that is reported by patients, and anxiety of the patient is a form of warning signal. It is a subjective phenomenon that causes a state of suffering which is connected with anxiety. Pain alerts against damage to the body, that is important to avoid injuries and also consequently for survival. Pain which is not caused by acute injuries, can be unpleasant for the patient, or it can change a person's life by reducing the quality of life. It also sometimes put an impact on the family of the patient. [2]

The time span of pain is a measurable characteristic which allows the differentiation between acute and chronic pain.
Based on duration and type, pain is classified into different categories as following:

Acute pain: persists < 3 months and it acts as a warning signal (post-operative pain, traumatic, associated with medical procedures).
Chronic pain: persists > 3 months and it does not fulfill the role of warning. It requires a multitherapeutic act.
Nociceptive Pain: It is due to direct stimulation of peripheral nerve endings (e.g burn,wound, fracture, angina)
Neuropathic Pain: It is because of the dysfunction of the pain perception system in the peripheral or central nervous system, which is a result of disease, injury or surgical damage (continuous experience of pain from an amputed limb-phantom limb pain).[3]

Thus, many diseases are having pain as a symptom that require treatment with analgesics.[4] Non- steroidal anti-inflammatory drug (NSAIDs) are widely used analgesics and can be used for any kind of acute or chronic pain. As they are both anti-inflammatory and analgesic, NSAIDs are the most widely used

therapeutic classes of drugs.[5] For severe pain currently, most potent pain-relieving drugs available are the Opioids. They have the broadest range of efficacy and provides the most reliable and effective method for pain relieving. But because of their dose related side effects like sedation, respiratory depression, constipation and addiction liability on long term use, their use is limited.

In India, herbs have always been the principle form of medicine. For e.g., Curcuma/turmeric have anti-inflammatory actions and is used as cough remedies, Aloe vera is used in various inflammatory diseases as well as skin diseases, Tulsi is administered against worms and parasites, insect poisoning. [6] Ficus Religiosa is a traditional medicinal plant found throughout India.[7]

It has significant nutritional constituents in all its parts. Fruits are rich in various macro and micronutrients like carbohydrates, protein dietary fibre, iron and calcium and are used in fresh as well as dried form. [8] The extracts of bark & leaf are being used in the traditional medicines. It has been reported that the stem bark of Ficus religiosa contains tannins, phenols, flavonoids, alkaloids, steroids, vit K, n-octacosanol, methyl oleanolate, lanosterol, β-sitosteryl-D-glucosides, stigmasterol, lupen-3-1.[7] All the parts (leaves, latex, bark and sap of root) have medicinal importance in our traditional system of medicine.[9]

In traditional Indian medicine, the stem bark is used extensively for the treatment of inflammation and glandular swelling of the neck. It is applied on ulcers and wound extensively and also used as a mouthwash to relieve toothache. Even though it is used in an extensive manner in traditional medicine as anti - inflammatory & analgesic agent[10,11], these effects have not been scientifically evaluated.

On the other hand, leaves are useful in regular diarrhea, asthma, toothache, astringent and, constipation.[9]These realities prompted the current study on the analgesic effects of this plant.[10] In the present study, we have evaluated the analgesic activity of methanolic extract of leaves and stem bark of Ficus religiosa. We have compared these activities between both the extracts which have not been done earlier. We have also performed the phytochemical screening of these extracts to confirm the presence of phytochemical substances and to study that whether they are responsible for the analgesic activity which we are going to evaluate.

Materials & Methods

The protocol was approved by the Institutional Animal Ethics Committee (IAEC) of KIMSDU, Karad. The study was conducted as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Experimental animals

Study was conducted in laboratory of pharmacology department of KIMSDU. Albino rats of either sex weighing 120-200gms bred in central animal house were

used. Animals were housed under standard conditions i.e with 12hr light and dark cycle. Temperature was maintained at **27-37 ºC**. They had free access to food and water up to the time of experimentation.

Drugs

Ibuprofen (Krishna Hospital Pharmacy), Methanolic extract of leaves and stem bark of Ficus Religiosa (prepared in KIMS Pharmacology Laboratory), Normal Saline were used.

Instruments used

Analgesic effect was tested by analgesiometer by tail flick method using radiant heat.Animals were divided into 6 groups.
Drugs were administered per orally (P.O)IBU: Ibuprofen
MEL: Methanolic leaves extract of Ficus religiosa, 100mg/kg (MEL100), 200mg/kg(MEL200)
MES: Methanolic stem bark extract of Ficus religiosa,125mg/kg (MES125), 250mg/kg(MES250)
The doses have been selected on basis of previous studies.[12,13]
Group1: 0.9% NS,10ml/kg, P.O
Group2: IBU, 40 mg/kg, P.O
Group 3: MEL, 100mg/kg, P.O
Group 4: MEL, 200mg/kg, P.O
Group 5: MES, 125mg/kg, P.O
Group 6: MES, 250mg/kg, P.O

In all groups the analgesic activity was tested before giving drugs i.e. 0 and after giving drugsat 30, 60 and 120 min after administration of drugs. [14] In order to prevent injury, 20 seconds exposure to radiant heat was taken as cut off points. 15

Statistical Analysis
All results were analyzed by one way ANOVA followed by Tukey Kramer post test.

Results

Table1
Mean and Standard Deviation of MEL100, MEL200, MES125, MES250 with control and Ibuprofen in rat tail flick method:

Time after treatment	Time of Tail flick					
	Control	IBU	MEL100	MEL200	MES125	MES250
0 min	9.666 ± 0.516	10 ± 0.894	9.66 ± 0.516	9.66 ± 0.516	9.83 ± 0.408	9.66 ± 0.516
30min	10.83 ± 2.137	19.33 ± 0.816	11 ± 1.414	14.33 ± 1.366	11.16 ± 0.752	14 ± 1.095
60min	11 ±	19.5 ±	13.33 ±	16.16 ±	14.66 ±	14.83 ±

	1.265	0.836	1.862	0.752	1.506	0.408
120min	9.5	16.83	10.16	12.83	10.33	12.5
	±	±	±	±	±	±
	1.378	1.329	0.752	2.041	1.033	1.871

Table 2
Effect of MEL100, MEL200, MES125 & MES250 treatments on Rat tail flick response compared with control

Time after treatment	Time of Tail flick					F value	P Value (Anova)
	Control	MEL100	MEL200	MES125	MES250		
0 min	9.666 ± 0.516	9.66 ± 0.516	9.66 ± 0.516	9.83 ± 0.408	9.66 ± 0.516	0.1351	0.9679
30min	10.83 ± 2.137	11 ± 1.414	14.33 ± 1.366 ##	11.16 ± 0.752	14 ± 1.095 ##	8.930	0.0001
60min	11 ± 1.265	13.33 ± 1.862 #	16.16 ± 0.752 ###	14.66 ± 1.506 ###	14.83 ± 0.408 ###	14.205	<0.0001
120min	9.5 ± 1.378	10.16 ± 0.752	12.83 ± 2.041 ##	10.33 ± 1.033	12.5 ± 1.871 #	6.012	0.0016
Repeated Measures ANOVA	F Value	2.021	25.83	147.93	47.39	106.54	
	P Value	0.1831	0.0001	<0.0001	<0.0001	<0.0001	

#, ##, ###: significant difference as compared with control group.

There was significant difference between the mean of MEL100, MEL200, MES125 and MES250 when compared to the control group. Post hoc tukey Kramer test suggests that there is significant difference between MEL200 and MES 250 at all time intervals when compared with the control group. It indicates that these are showing analgesic activity at all time intervals while MEL 100 and MES125 are showing analgesic activity at 60 min only.
Repeated measures ANOVA shows that there is significant difference in the analgesic activity of all the test drugs at their respective time interval.

Table 3
Effect ofMEL100, MEL200,MES125 and MES250 treatments on Rat tailflick response compared with IBU

Time after treatment		Time of Tail flick					F value	P Value (Anova)
		IBU	MEL100	MEL200	MES125	MES250		
0 min		10 ± 0.894	9.66 ± 0.516	9.66 ± 0.516	9.83 ± 0.408	9.66 ± 0.516	605.29	<0.0001
30 min		19.33 ± 0.816	11 ± 1.414 ***	14.33 ± 1.366 ***	11.16 ± 0.752 ***	14 ± 1.095 ***	54.259	<0.0001
60 min		19.5 ± 0.836	13.33 ± 1.862 ***	16.16 ± 0.752 ***	14.66 ± 1.506 ***	14.83 ± 0.408 ***	23.105	<0.0001
120 min		16.83 ± 1.329	10.16 ± 0.752 ***	12.83 ± 2.041 ***	10.33 ± 1.033 ***	12.5 ± 1.871 ***	19.669	<0.0001
Repeated Measures ANOVA	F Value	301.23	25.83	147.93	47.39	106.54		
	P value	<0.0001	0.0001	<0.0001	<0.0001	<0.0001		

***: significant difference as compared with the ibuprofen group.

There was significant difference between mean of all test drugs when compared with the ibuprofen by one way ANOVA. Following post hoc tukey Kramer, all test groups have significant difference when compared with the ibuprofen which suggests that analgesic activity of all test groups are not comparable with the ibuprofen. Between the two doses of leaf extract, we found MEL200 is more effective while between the two doses of stem extract, MES 250 has shown more analgesic activity. Between MEL200 and MES 250, it was found that MEL200 has shown more analgesic activity though no significant difference was found($p>0.05$), following post hoc Tukey Kramer. Repeated measure ANOVA indicates significant difference in all test drugs at different time interval.

Table 4
Result of Phytochemical screening of methanolic extract of leaves and stem bark of Ficus Religiosa

	Leaves extract	Stem bark extract
Carbohydrates	+	+
Steroid	+	+
Terpenoids and their Glycosides	+	+
Flavonoids	**++**	+
Alkaloids	--	**+++**
Phenolic compound and tannins	**++**	**++**

Discussion

Pain is the most common episode experienced by the patients and may be considered as a form of warning signal of any pathological state. [16] In recent decades, drugs like aspirin and morphine have been used widely. In most cases, these drugs can relieve 50% of pain in almost 30% of patients. Along with this, they cause serious side effects. Various studies shown that opiates tend to cause physical dependency, addiction & tolerance whereas NSAIDs are more likely to cause gastrointestinal disorders.[17] Herbs have always been the principle form of medicine. They have curative properties due to various complex chemical substance of different composition. [11]

In the present study, analgesic effects of different doses of methanolic extract of leaves and stem bark of *F. religiosa* were tested in experimental model of pain. Both the extracts have produced analgesic activity when compared with the control group. It was observed that both doses of methanolic extract of leaves (100, 200mg/kg) have shown analgesic activity when compared with the control group. Our results coincide with the results of Vishal Gulecha et al (methanolic extract of leaves at 20, 40mg/kg) in which they have shown significant analgesic activity at 40mg/kg. [13]

Similarly, both doses of methanolic extract of stem bark (125, 250mg/kg) have shown analgesic activity when compared with the control group. D.R Marassini et al has conducted one study in which they have used ethanolic extract of bark and leaves of ficus religiosa (200, 400mg/kg) for evaluating analgesic activity by Eddy's hot plate method and acetic acid induced writhing test in mice models. They have also observed significant analgesic activity in these extracts. [9] In the study of R. Sreelaxmi et al, they observed significant analgesic activity of both the leaves and stem bark methanolic extract (250, 500mg/kg) in acetic acid induced writhing test in mice. They have also reported that the stem bark of ficus religiosa contain tannin andlikely this is responsible for its analgesic activity. [12]

In this study, we have compared the leaves extract and stem bark extract for their analgesic activity. We have also compared the two different doses of leaves extract i.e 100mg/kg (MEL100) and 200mg/kg (MEL200) with each other for analgesic activitiy. Similarly, we have compared two different doses of stem bark extract i.e 125mg/kg (MES125) and 250mg/kg (MES250) with each other for their analgesic activity. This comparision of different doses of the extract has not been done earlier.

In case of analgesic activity, we observed that among both doses of leaves extract i.e 100 mg/kg (MEL100) and 200mg/kg (MEL200), MEL200 has shown more analgesic activity (Table 3). Similarly in case of stem bark extract, among both doses i.e. 125mg/kg (MES125) and 250mg/kg (MES250), MES250 has shown more analgesic activity. When we compared MEL200 and MES 250, they have shown similar analgesic activity. (Table3). So, we conclude that while comparing the analgesic activity, both leaves and stem bark extract i.e. MEL200 and MES250 have shown similar analgesic activity.

We have done phytochemical screening for both the extracts and we observed the presence of tannins, flavonoids, sterols, phytosterols in both the extracts while presence of alkaloids was found only in the stem bark extract. The presence of phytochemical constituents like tannins, flavonoids, steroids, alkaloids may be the basis of this activity. Analgesic effects of flavonoids, steroids, and tannins have been reported earlier in the study done by Vishal. Gulecha et al. [13] R.Sreelaxmi et al also have reported that the stem bark of ficus religiosa contain tannin and likely this is responsible for its analgesic activity. [12]

Conclusions

Methanolic extract of leaves as well as stem bark of Ficus Religiosa have shown analgesic effect at different time intervals when compared with the control group. Both the extracts have shown more analgesic activity at their respective higher doses. When we compared the analgesic activity of both the leaves and stem bark extract, both have shown similar activity. But we have also observed that their analgesic activities were not comparable with the standard drug ibuprofen. The results of our study revealed that the leaves and stem bark extract of ficus religiosa have analgesic activity, which needs to be investigated further.

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