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ASSESSMENT OF PHARMACOLOGICAL ACTIVITIES OF AERIAL ROOT OF *FICUS BENGHALENSIS* LINN.

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ABSTRACT

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. Over 50% of all modern clinical drugs are having their origin in natural products. Presently 80% of the world population relies on plant derived medicines and serves as first line of defense in maintaining health and combating many diseases. In the present study we assessed several pharmacognostic activities as antispasmodic, muscle relaxant, acute toxicity, analgesic, tail immersion and paw reading activities of aerial roots of *Ficus benghalensis* Linn. An aerial root extracts in different solvent Ethanol, Methanol and Distilled water was applied on albino mice. The analgesic activity was done by using acetic acid induced activity, Tail immersion and Paw readings was done with radiant heat hot plate method. All the three types of extracts showed excellent potential against analgesic activity, Tail immersion and Paw readings activities at 200 mg/kg dose as compare to the standard drug by applying on animals albino mice. We found that aerial root showed antispasmodic potential through active charcoal movement about 9.33±1.52*, 13.33±1.52* and 12.66±1.52* at 200mg/kg dose of using methanol, ethanol and distilled water extracts respectively. We concluded that aerial root extracts in Ethanol, Methanol and Distilled water of *Ficus benghalensis* Linn, showed excellent potential against Antispasmodic, Muscle relaxant and Acute toxic activities at 200 mg/kg dose as compare to the standard drug applied on animals albino mice. Ethanolic

extract of aerial root of *Ficus benghalensis* Linn. was found to be more potent and effective out of the three extracts in each activity and was dose dependent. Further research is needed to explore anti-tumer, anti diarrheal activities of *Ficus benghalensis.*

KEYWORDS: *Ficus benghalensis* Linn. Aerial root, Analgesic, Antispasmodic, Acute toxic, Muscle relaxant, Tail immersion, Paw reading activities.

INTRODUCTION

Plants are an essential component of the universe. After various observations and experimentations many medicinal plants were identified as source of important medicine (Malik etal., 2001). Medicinal plants have been used since prehistoric period for the cure of various diseases. Since these are in common use by the local people and are of great importance that's why a lot of people are engaged in the trade of important medicinal herbs throughout the world. Especially, people living in villages have been using indigenous plants as medicines (Qureshi and Khan., 1971).

Knowledge of medicinal values of plants is recognized by almost every society on earth. The inhabitants of the remote places have good knowledge about the utilization of plants because of the non-availability of synthetic drugs. In addition, for the survival, they use the plant-based drugs growing nearby their villages. Based on their right or wrong experiences they discovered the therapeutic agents of these plants in particular diseases. These experiences are transferred from parents to offspring (Qureshi, 2004). In nearly every country of the world, treatment through herbs and some traditional medicine system is progressing. In Indo-Pak. Subcontinent, these traditional systems are called unani or ayurvedic system (Malik etal., 2001).

Medicinal plants have served through the ages, as a constant source of medicaments for the exposure of variety of diseases. The history of herbal medicine is as old as human civilization. The plants are known to provide a rich source of botanical anthelmintics, antibacterials and insecticides (Satyavati etal., 1976). Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show

immense potential of medicinal plants used in various traditional systems. Today, we are witnessing a great deal of public interest in the use of herbal remedies (Arulmozhi and Sathiya, 2007).

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value (Nostro etal., 2000). The development of microbial resistance to antibiotics has led the researches to investigate the alternative sources for the treatment of resistant strains (Hammer etal., 1999). Presently 80 percent of the world population relies on plant derived medicines and serves as first line of defense in maintaining health and combating many diseases (Veale etal., 1992).

However, their scientific study has been made possible only after the development of microbiology. Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms (Gordon 2001). Over 50% of all modern clinical drugs are having their origin in natural products (Suffness and Douros 1982). In general, bacteria have the genetic ability to transmit and acquire resistance which is utilized as therapeutic agents (Cohen 1992). The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Nostro etal., 2000).

Phytochemicals are naturally occurring in the medicinal plants leaves, stem bark, fruits and roots that have defense mechanism and protect from various diseases. Natural products from plants called secondary metabolites are the end products of primary metabolites such as carbohydrates, amino acid, and chlorophyll lipid so on. They are synthesis large variety of chemical substances known as secondary metabolites which include alkaloids, steroids, flavonoids, terpenoids, glycoside, saponia, tannins, phenolic compounds etc. (Ghahi 1990). The active principles of many drugs found in plants are secondary metabolites. (Dobelis 1993). Therefore basic phytochemical investigation is vital. The identification and isolation of such active compounds makes it more effective therapeutic application. It present consumes from taking certain plants that have no medicinal value or poisonous to them. It will lead to better understanding of diseases Moringa pterygosperma Gaertn (Moringaceae), native to the western and sub Himalayan region, India, Pakistan, Africa and Arabia is now distributed in the Philippines, Cambodia, Central North and Caribbean Island (Morton 1991).

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Davis, 1994). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions (Ahmad etal., 1998). This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance (Monroe and Polk, 2009) .There is a constant need for new and effective therapeutic agents (Bhavnani, and Ballow, 2000). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Cordell, 2000).

MATERIAL AND METHODS

Collection of plants materials

Fresh and healthy aerial roots of *Ficus benghalensis* were collected from Peshawar in February 2017 and were identified by taxonomist Prof. Gillani Department of Botany, University of Peshawar.

Sample preparation

Collected aerial root parts and were dried under shade at room temperature then were grind by the help of coffee grinder (Sunbeam EM0415) and sifted to assure particles where <1mm particle size fresh and dried *Ficus benghalensis* aerial roots were promiscuous sampled for moisture content tenacity.

Solvent used

In present study three different solvents ethanol, methanol and distilled water were used to determine the extract indicates high pharmacological properties of *Ficus benghalensis*. The aerial root powder about 30g weight by fisher Scientific scale were dispensed to each flask. The flasks were kept for 5 days at room temperature for well dissolution of solvents in plant materials. After 5 days the solvents were filtered through Whatmman filter paper No.01. The filtrate were putted and passed through Rotary Vacuum Evaporator (HAHNSHIN scientific Co, South Korea). After Rotary the crude extracts was dispensed to the china dish and was kept in water bath for the complete purification of extract from solvents and then utilized in sterile vials.

Animals: Albino mice for various studies were obtained from NIH (National Institute of Health) Islamabad. Weights of the mice were ranged from 20-25g.

Analgesic activity

Procedure

Analgesic activity was checked by albino mice for aerial root extract with three replicates of each concentration (100, 150, 200mg/kg) and divided the 18 mice in 6 groups this procedure was applied for each extract (aerial root). 50mg/kg of diclofenic sodium was injected to one group for the positive control and for negative control 10ml/kg of acetic acid to another group of mice. Before one hour starting the activity the supply of food was stoped to the animals. In third, four and fifth group of mice was given methanolic extract (100, 150, 200mg/kg). After injection of the plant extract, saline and diclofenic sodium, 1 percent of acetic acid induced pain in peritoneal cavity of remaining group of mice. After injecting the acetic acid the mice started writhing and was counted for next 10 minute and the percent result expressed inhibition (Akuodor *et al*., 2011).

{(A-B)/A} X 100 = % inhibition

Where, B= Average number of writhing of the test group.

A= Average number of writhing of the control group.

Tail Immersion Test

Procedure

The control analgesic activity was assessed by tail immersion test in mice. Mice were divided into groups of six animals each. The lower portion 5 cm of the tail was immersed in a beaker of water maintained at 55±0.5℃. The time in second for tail withdrawal from the water was taken as the reaction time with a cut off time of immersion at 10sec. The reaction time was measured 1 hour before and after oral administration of extracts (10, 15 and 20mg/kg, per oral) or distilled water (10ml/kg), Ibuprofen (100mg/kg) was administrated subcutaneously 30min before the test

Paw readings

Procedure

Mice were divided into three groups of each. The first group served as control and received normal saline (0.1ml/ 10 g p.o.). The second group was administered ibuprofen (100 mg/kg) as the standard drug and third group received ethanolic, Methanolic and distilled water extract of aerial roots at dose of 10,15 and 20 mg/kg. aerial root having extract showed active charcoal movement about 18.33±2.08*, 18±1.73*and 18.66±1.15* at 200mg/kg. The test was carried out using hot plate apparatus, the temperature was set at 55±1°. Mice were placed

on hot plate and recorded the reaction time in second for licking of hind paw or jumping with cut off time of 15 s. The reaction time following the administration of the test extracts, reference standard drug, and control saline vehicle were measured at 0-60 sec.

Antispasmodic activity

Procedure

Weighing 20-30 g of 15 mice for aerial root with three replicate of each concentration (100, 150, 200mg/kg) the 15 mice divided into five groups and before experiment gives no food to mice. Give castor oil (10ml/kg) orally to the mice for negative control in one group and saline water (10ml/kg) for the positive control to another group of mice.1ml of charcoal gives orally to every group of mice this charcoal is used for the identification of movement in mice. And remaining three groups of mice give the plant extract (100, 150, 200mg/kg) through injection. The distance cover by the charcoal from pylorus to caecum can be measure after 50 min of giving charcoal to express the distance percent travel by charcoal to the intestinal length ratio. The extract percent inhibition can be calculated that can produce (Manohar *et al*., 2009).

Acute Toxicity Test

Procedure

Mice were used for each extract of aerial root and these extract was injected directly into the mice with different concentration 100, 150, 200mg/k. After injecting the extract death of the animals were recorded to find either the extract is acute toxic or having safe potency (Uddin *et al*., 2014).

Muscle Relaxant Activity

Procedure

In this activity 15 mice were used for aerial root extract with 3 replicates of each concentration 100, 150, 200mg/kg and these 15 mice for each extract was divided into five groups. The distilled water (10ml/kg) were used for negative control in 1 group of mice and for positive diazepam (1mg/kg) was used in other group. The remaining group was treated with plant extract (100, 150, 200mg/kg). The wire was attached with stand about 60cm above from the ground. After treatment the animal were exposed for muscle relaxant test. By hind legs each mice were hanged on the wire and recorded the hanging time. When the mice were failed to hang on wire for 5s was due to the presences of muscle relaxant activity (Muhammad *et al*., 2013).

Statistical analysis

The results of this activity were carried out using one-way ANOVA by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group *P <0.05 and **P <0.01 were considered to be statistically significant.

RESULTS

Phytochemical screening

Preliminary phytochemical analysis of the methanol extracts of the root of *F. religiosa* and *F. benghalensis* showed the presence of carbohydrates, flavonoids, amino acids, steroids, saponins and tannins.

Analgesic activity of Aerial root of *Ficus benghalensis*

The aerial root having distilled water extract showed No. of writhings 10.33±0.57, 8.33±0.57, 7.66±0.57 at 100, 150, 200mg/kg. While ethanol extract showed 14±2.64, 9±1, 7±1 no of writhing's at 100, 150 and 200mg/kg. Similarly the methanol extract of aerial root exposed 21.33±1.52, 16.66±1.52 and 14±1 at 100, 150, 200mg/kg. The whole extract of aerial roots bared significant result parallel to standard drug Diclofenic sodium having 19±1 No.of writhing's at 10mg/kg.

Table I: Analgesic activity of aerial root of *Ficus benghalensis.*

Treatment	Dose	No. of writhing	% inhibition
Normal Saline	10ml/kg	72±1	
Diclofenic sodium	10mg/kg	19±1	45
ARDWE	100mg/kg	10.33±0.57	57
	150mg/kg	8.33±0.57	60
	200mg/kg	7.66±0.57*	61
AREE	100mg/kg	14±2.64	52
	150mg/kg	9±1	59
	200mg/kg	7±1*	62
ARME	100mg/kg	21.33±1.52	42
	150mg/kg	16.66±1.52	49
	200mg/kg	14±1	52

Tail immersion activity of Aerial root of *Ficus benghalensis*

The aerial root having distilled water extract showed time interval having Tail immersion test 1.44±0.04, 1.56±0.03, 1.89±0.02 at 10, 15, 20 mg/kg. While ethanol extract showed 1.76±0.02, 1.86±0.01, 1.94±0.02 at 10, 15, 20 mg/kg. Similarly the methanol extract of aerial root exposed 1.44±0.04, 1.56±0.03 and 1.89±0.02 after 60 second time interval at 10, 15, 20

mg/kg. The whole extract of aerial roots bared significant result parallel to standard drug Ibuprofen having 2.95±0.02 having time interval at 10mg/kg.

Table II: Tail immersion activity of Aerial root of *Ficus benghalensis.*

SNO	TREATMENT	DOSE	0 SEC	60 SEC
1	**Normal saline**		0.75±0.01	0.78±0.01
2	**Ibuprofen**	100 mg/kg	0.83 ±0.01	2.95±0.02
3	ARDWE	10 mg/kg	0.77±0.03	1.44±0.04
4		15 mg/kg	0.78±0.02	1.56±0.03
5		20 mg/kg	0.80±0.01	1.89±0.02
6	AREE	10 mg/kg	0.76±0.02	1.76±0.02
7		15 mg/kg	0.79±0.03	1.86±0.01
8		20 mg/kg	0.83±0.02	1.94±0.02
9	ARME	10 mg/kg	0.76±0.02	1.44±0.04
10		15 mg/kg	0.79±0.03	1.56±0.03
11		20 mg/kg	0.80±0.02	1.89±0.02

Paw readings of Aerial root of *Ficus benghalensis*

The aerial root having distilled water extract showed time intervals 1.55±0.1, 1.73±0.05 and 2.03±0.05 at 10, 15, 20mg/kg. While ethanol extract showed 1.44±0.04, 1.56±0.03 and 1.89±0 at 10, 15, 20mg/kg. Similarly the methanol extract of aerial root exposed 02 1.32±0.03, 1.68±0.02 and1.99±0.01 at 10, 15, 20mg/kg. The whole extract of aerial roots bared significant result parallel to standard drug **Ibuprofen** having 2.95±0.02 time interval at 100 ml/kg.

Table III: Paw readings activity of aerial root of *Ficus benghalensis.*

SNO	TREATMENT	DOSE	0	60 SEC
1	**Normal saline**		0.75± 0.01	0.78±0.01
2	**Ibuprofen**	100 ml/kg	0.83 ± 0.01	2.95±0.02
3	ARDWE	10 mg/kg	0.76± 0.02	1.55±0.1
4		15 mg/kg	0.79± 0.03	1.73±0.05
5		20 mg/kg	0.80± 0.02	2.03±0.05
6	AREE	10 mg/kg	0.78±0.03	1.32±0.03
7		15 mg/kg	0.79±0.02	1.68±0.02
8		20 mg/kg	0.82±0.01	1.99±0.01
9	ARME	10 mg/kg	0.77±0.03	1.44±0.04
10		15 mg/kg	0.78±0.02	1.56±0.03
11		20 mg/kg	0.80±0.01	1.89±0.02

Antispasmodic activity of Aerial root of *Ficus benghalensis*

The aerial root having distilled water extract showed antispasmodic activity having charcoal movement about 2.33±057, 5.66±1.52, 9.33±1.52* at 100, 150, 200mg/kg. While ethanol

extract showed 4.66±1.52, 8±1, 13.33±1.52* at 100, 150, 200 mg/kg. Similarly the methanol extract of aerial root exposed 5±1, 8±1and 12.66±1.52* at 100, 150, 200mg/kg. The whole extract of aerial roots bared significant result parallel to standard control having 12±1 distance covered at 10ml/kg.

Table IV: Antispasmodic activity of aerial root of *Ficus benghalensis*.

Treatment	Dose	Total Length Of Intestine	Distance Covered By Charcoal	% distance covered by charcoal
Control	10ml/kg	57±1	12±1	35
Castor oil	10ml/kg	52±1	29±1	28
ARDWE	100mg/kg	45±6	2.33±057	7
	150mg/kg	48±2	5.66±1.52	35
	200mg/kg	49±1	9.33±1.52*	65
AREE	100mg/kg	53±1	4.66±1.52	26
	150mg/kg	53±2	8±1	54
	200mg/kg	54±1	13.33±1.52*	99
ARME	100mg/kg	51±1	5±1	29
	150mg/kg	50±3	8±1	54
	200mg/kg	50±4	12.66±1.52*	93

Acute Toxicity Activity of Aerial root of *Ficus benghalensis*

The aerial root having distilled water extract showed % mortality is 0, 0 and 60 % mortality at 100, 150, 200mg/kg. While ethanol extract showed 0, 0 and 60 % mortality at 100, 150, 200mg/kg. Similarly the methanol extract of aerial root exposed 0, 0 and 60 % at 100, 150, 200mg/kg. The whole extract of aerial roots bared significant result parallel to standard drug Normal saline having 100% mortality at 10ml/kg.

Table V: Acute toxicity activity of aerial root of *Ficus benghalensis*.

Treatment	Dose	No. of animals died/5	% mortality
Normal saline	10ml/kg	5/5	100
ARDWE	100mg/kg	0/5	0
	150mg/kg	0/5	0
	200mg/kg	3//5	60
AREE	100mg/kg	0/5	0
	150mg/kg	0/5	0
	200mg/kg	3//5	60
ARME	100mg/kg	0/5	0
	150mg/kg	0/5	0
	200mg/kg	3//5	60

Muscle Relaxant Activity of aerial roots of *Ficus benghalensis*.

(Traction Test)

The aerial root having distilled water extract showed time interval having traction test is 13.66±1.15, 9±2 and 4±2 at 100, 150, 200mg/kg. While ethanol extract showed 11±2, 7±2 and 3.33±0.57 at 100, 150, 200mg/kg. Similarly the methanol extract of aerial root exposed 10.33±1.15, 7±2 and 3.33±0.57 at 100, 150, 200mg/kg. The whole extract of aerial roots bared significant result parallel to standard drug Diazepam having 99.66±0.57 time interval at 1mg/kg.

Table VI: Muscle relaxant activity of aerial root of *Ficus benghalensis*

Groups	Dose	Traction test
		30 min
Distilled water	10ml/kg	0±0.00
Diazepam	1 mg/kg	99.66±0.57
ARDWE	100mg/kg	13.66±1.15
	150mg/kg	9±2
	200mg/kg	4±2
AREE	100mg/kg	11±2
	150mg/kg	7±2
	200mg/kg	3.33±0.57
ARME	100mg/kg	10.33±1.15
	150mg/kg	7±2
	200mg/kg	3.33±0.57

DISCUSSION

Similar work was reported by many workers as Vishnu et al., (2010) conducted analgesia activity of *Ficus benghalensis* and showed significant analgesic effect at higher dose. Sreelekshmi et al., (2007) carried out analgesic activity and reported that *Ficus Regligiosa* has analgesic potential which support our result. Vishnu et al., (2010) reported the analgesic effect of Stem bark extraction of *Ficus benghalensis* Linn. Sreelekshmi et al., (2007) conducted analgesic studies on stem bark of *Ficus religiosa* Linn. These all worker carried out the similar studies which strengthen the present finding.

We have studied many research articles regarding pharmacological activities of genus *Ficus* but no previous work has been done by any researcher on muscle relaxant activity of *Ficus benghalensis* L. Therefore more research is needed on *Ficus benghalensis* and identifies its muscle relaxant based activity. Wiktin et al., (1961) conducted analgesic activity of *Ficus benghalensis*. Most of the researcher conducted experiments and reported that analgesic activity of different medicinal plants produced significant effects against the test species,

which support our result. Vikas et al., (2010) carried out analgesic activity of *Ficus benghalensis* and showed significant result similar to our findings. Otimenyin (2004) also reported the analgesic effects of the plant in central pain using the hot plate test method in mice Prabhakar et al., 1981 disclosed that vitexin (8-C-glucosyl apigenin) and isovitexin (6-C-glucosyl apeginine) have several pharmacological activities such as anti-hypertensive, anti-inflammatory, antispasmodic, antimicrobial and antioxidant. Hence, these compounds have been selected as analytical markers to standardize aqueous and methanol extracts of leaves of three verities of *Ficus deltoidea*. *F. thonningii* has been reported to possess analgesic properties that are comparable to aspirin in both peripheral and central induced pain. Using the acetic acid induced writhing reflex model in mice.

Zulfikar et al., 2010 investigated the analgesic activity of crude extracts of *F. racemosa* respectively. The extracts of plants were found to exhibit a dose dependent increase in latency time when compared with control. At 90 minutes, the percent inhibition of two different doses (100 and 200 mg/kg body weight) was 50.14% & 56.56% for *F. racemosa* respectively. The results were found to be statistically significant ($p<0.001$). Similar work was performed by various researchers like Vikas *et al*., 2010 conducted analgesic activity of *Ficus benghalensis* Linn. (*Moraceae*). Otimenyin *et al*., (2004) demonstrated that methanolic extracts of *F. thonningii* (500 mg/kg) administered intraperitonially had a percentage inhibition (79.7%) comparable to aspirin (80%) showing that *F. thonningii* has analgesic effects that can be useful in the management of peripherally induced pain.

CONCLUSION

There is no doubt that additional studies are required to characterize the chemical components and to find out more pharmacological activities of *F.bengalensis.* The present literature had shown that the *Ficus benghalensis* L. have higher value in the traditional and folk medicines. So in this study aerial root extracts of *Ficus benghalensis* L. were considered to determine the analgesic, tail immersion and paw reading, antispasmodic, muscle relexant and acute toxicity activities of the tested plant. The present studies revealed the presence of potential capability of plant in prescribe activities compare with standard drugs. Our results provides scientific experimental support for the medicinal use of aerial roots as pain killer, antispasms. Toxicity depend on dose (200mg/kg) and muscle relaxant agents.

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