ORIGINAL ARTICLE OPEN ACCESS

Investigation of the Laxative Activity of Methanolic Extract of Tamarix Aphylla L. Karst (Saltcedar) In Experimental Animals

Khushal Khan, Niaz Ali, Nawaz Sharif, Rooh Ullah, Tauseef Ahmad, Mohsin Ali ****

Department of Pharmacology, Institute of Basic Medical Sciences, Khyber Medical University Peshawar

Phytomedicines present potential uses because pharmacological activities of different herbs have not been explored completely. Tamarix aphylla (L.) Karst, mentioned in the Holy Quran, is one amongst different plants used traditionally as laxative. Keeping in view the traditional use of Tamarix aphylla as laxative, current work was carried out to determine its use on scientific grounds. The methanolic extract of Tamarix Aphylla (L.) Karst was prepared. The lethal dose (LD50) was calculated. Charcoal meal gastrointestinal transit test and weight of the faeces matter were used for evaluation of the laxative potential of the extract. For charcoal meal gastrointestinal transit test, mice were divided into four groups of six animals each, the first group as a negative control, second group as a positive control (Neostigmine) while group third and fourth were treated with methanolic extract of Tamarix Aphylla at doses of 150mg/kg, 300mg/kg and 450mg/kg p.o. respectively. For the weight of the faecal matter method, rats were divided into five groups of six animals each, the first group as a negative control, second group as a positive control (Neostigmine) while group third, fourth and fifth were treated with methanolic extract of Tamarix Aphylla at doses of 150mg/kg, 300mg/kg and 450mg/kg p.o. respectively. The series of experiments showed that the LD50 for the methanolic extract of Tamarix Aphylla in animal models was 1650mg/Kg. A significant increase in movement of the charcoal meal was produced at 300mg/kg dose of extract when compared to the normal control group (p-value <0.05). In weight of the faeces matter method, the faecal output of rats was increased significantly at doses of 300 and 450 mg/kg when compared to the normal control group (p-value <0.05). The results proved that the methanolic extract of Tamarix Aphylla (L.) Karst has a significant laxative activity.

Keywords: Tamarix Aphylla; Constipation Laxative; Charcoal Meal; Senna glycosides

INTRODUCTION

Constipation is known as one of the most frequent gastrointestinal disorders. Constipation affects a patient's quality of life in chronic conditions. It is more prevalent in women and adults of age (65 & above) than in children. The prevalence of constipation varies from 2-27% (1). Besides the decrease in gastric motility, dietary pattern, and lifestyle also influences gastrointestinal motility. Lack of fibre in diet, sedentary lifestyle, insufficient liquid intake, consumption of certain medicines, and changes in routine etc are the major factors responsible for causing constipation. Often mild and intermittent symptoms are associated with constipation but may become chronic, debilitating, and difficult to treat with progress. Inguinal hernia and hepatic encephalopathy are some long-term complications of constipation (2). A study conducted in Fatima Hospital Karachi, Pakistan, revealed that 22% of inguinal hernia patients had chronic constipation (3). Treatments available for chronic constipation have varying degrees of efficacy. Therapeutic options available include stool softeners, fibre supplements, osmotic and stimulant laxatives, and the secretagogues lubiprostone and linaclotide (4). A higher prevalence of laxative use in the elderly was demonstrated in an epidemiologic study. Similarly, elderly patients living in nursing homes have a much greater prevalence of constipation (up to 50%), with up to 74% of them using daily laxatives (5).

Herbal medicines are becoming popular even in this generation where everything is governed by science. Healing with medicinal plants predates written human history as archaeological evidence indicates that the use of medicinal plants dates at least to the Palaeolithic, approximately 6,000 years ago (6). Plant's medicinal value is due to the presence of phytochemicals that produce physiological and pharmacological actions in the body (7). Plants have long been a very important source of drugs against several diseases including constipation (8). One of the commonly used medicinal plants in botanical medicine for constipation is *Tamarix aphylla.* Its local names i.e. Athel tree, Athel tamarisk, Athel pine, and salt cedar (9). *Tamarix aphylla* is an evergreen tree and native to East, North and Central Africa. Also found in some parts of Southern, Western and Central Asia and the Middle East, Arabian Peninsula, Afghanistan, Iran and India (9). The taxonomical Position of *Tamarix aphylla* is shown in **Table 1** (10). It is a rapidly growing plant, and it has firm branches, round or irregular spreading crowns and long loose twigs. Its height ranges from 12 to 60 feet (11 -18 m) and its trunk diameter is about 2.5 feet (approximately 0.8 m). The plant has small leaves, scaly in shape and 0.05 12-inch (1.5 mm) long. The twigs are thin, slender, and connected. It has narrow ridges on the trunk and the bark is thick, flat on the branches. The stem is brownish red, curved, slender and branched with permeable sapwood. Flowers are Pinkish, bisexual with a precise short stalk. Tannins are present in all extracts. Cardiac glycosides, steroids, flavonoids, and terpenoids were the other metabolites found. No saponins and cyanogen glycosides were found (11).

Tamarix aphylla is used as a herbal medicine for a variety of indications such as diuretic, carminative, anti-inflammatory and treatment of internal hematomas (12) tuberculosis, leprosy, smallpox, eczema and other skin diseases (13). On the other hand, it has cardio-protective effects in Doxorubicin-induced toxicity and is scientifically proved as an analgesic and antipyretic, antimicrobial and antifungal activity (14) (15) (16) (17). There is a paucity of scientific information on the laxative of *Tamarix Aphylla* despite the extensive use in ethnomedicine for this purpose

(18). The current study was designed to evaluate the laxative activity of leaf extracts of *Tamarix Aphylla* in animal models.

Table 1: The taxonomical Position of *Tamarix aphylla*

Taxonomy of *Tamarix aphylla*
Kingdom: Plantae
Unranked: Angiosperms
Unranked: Eudicots
Order: Caryophyllales
Family: Tamaricaceae
Genus: Tamarix
Species: aphylla

Table 2: Phytochemical compounds identified in different extracts

Metabolite	**Aqueous-Extract**	**Aqueous-Ethanol Extract**	**Aqueous-Methanol. Extract**	**Aqueous-Acetone. Extract**
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Alkaloids	-	-	-	-
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Cardiac glycosides	Trace	+	+	+
Cyanogens glycosides	-	-	-	-
Free quinines	-	-	-	-
Anthraquinones	+	-	-	-
Saponins	-	-	-	-
Reducing sugar	+	-	-	-
Gum	Trace	-	-	-

MATERIAL & METHODS

The study was carried out in the Department of Pharmacology, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Khyber Pakhtunkhwa, Pakistan. The Animal Ethics Committee, IBMS, Khyber Medical University, Pakistan guidelines were followed for carrying out the experimental study. Ethical approval was obtained from the Animal Ethics Committee (**DIR/KMU-AS&RB/LA/000491).** The highest purity grade chemicals i.e., neostigmine and Senna (sennoside) were used as a reference. Both chemicals were obtained from the local chemical industries in Pakistan. Acacia powder, vegetable charcoal and hydrolysed starch were used for the charcoal meal gastrointestinal transit test. Material used were of analytical grade. For all chemicals stock solutions were made in distilled water and on the day of activity fresh dilutions were made in normal saline. Thirty male Wistar albino rats weighing 180-200gm and age 8-10 weeks older were used for the conduction of experiments in the current study. Rats were acquired from the National institute of health (NIH) Islamabad, Pakistan and were then held in an appropriately ventilated room with optimum temperature (25°C ± 2°C) and moisture (40-50%) in 12hrs light-dark cycles a day. Rats were held in a ventilated room for two weeks before going to conduct the experiment and were fed with balanced laboratory prepared food and free water access was allowed to them.

Collection of Plant and Extraction:

In April 2017, *Tamarix aphylla* L. Karst tree leaves were obtained from the Botany Department, University of Peshawar, Pakistan. The plant was recognized and botanically validated by the Head of the Department. A coupon sample (No.20089 PUP) was given at the herbarium of the department. Shade drying of the leaves was carried out at 30-35℃ and then crushed into a fine powder with the help of auto -mix blender. The powder was then soaked at room temperature in 5L/kg of 70 % methanol for two weeks with regular stirring and mixing. The filtrate was then exposed to reduce pressure at 40 C using a rotary evaporator for concentrated extract. Around 221.47 g, 6.61% w/w of a green soluble crude residue was obtained. Normal saline was used for the dissolution of the extract.

Measurement of Lethal Dose (LD_{50})

To calculate LD_{50} of *Tamarix aphylla* leaves extract, normal healthy mice were placed into extract-treated "test" groups and normal saline-treated "control" group (6 mice/group). In each of the test groups, a dose of (250, 500, 750, 1000, 1250, 1500, 1600, 1650 1800, 2100, 2400, 2700 and 3000 mg/kg) of *Tamarix aphylla* extract was administered intraperitoneally to every mouse in the group. In the control group, normal saline was administered to individual mice (19).

Effect of Tamarix aphylla on Charcoal meal gastrointestinal transit:

For this experiment, some small changes were done in the method of Croci et al. (20). Four groups of mice were made each containing six animals (**Table 3**). The first group was labelled as negative control (normal saline: 10mL/kg, p.o), the second group was labelled as the positive control (Neostigmine:1 µg/kg, i.p.) while group third and fourth labelled as extract groups were treated with methanolic extract of *Tamarix Aphylla* at doses of 150mg/kg and 300mg/kg p.o. respectively. As per protocol 15 minutes after dosing, 0.3 mL of charcoal meal (distilled water suspension containing 10% vegetable charcoal, 10% gum acacia, and 20% starch) was then given orally to

each mouse. Animals were then dissected after 30 minutes, and the complete small intestine was removed. The laxative activity was calculated by measuring the distance travelled by the charcoal meal in the small intestine and then percentage was calculated with respect to the total length of the small intestine.

Table 3: Division of Animal models into groups (n =24)

S.No	Group	Type	Drug Administered
1.	Group-I	Negative control	Normal Saline (NaCl)
2.	Group-II	Positive Control	Neostigmine in NaCl
3.	Group-III	Extract Group-I	150mg/Kg
4.	Group-IV	Extract Group-II	300mg/Kg

Effect of Tamarix aphylla on Weight of the faeces matter:

For calculation of laxative activity, the method of Capasso *et al*. was followed. Before starting the experiment, rats were fasted for 12 hours and then were kept in separate cages specially designed for this experiment. Rats were divided in five groups six in each (**Table 4**). The first and second groups were termed as negative and positive control and received normal saline (5ml/kg, P.O.) and Senna (10mg/kg, P.O) respectively. The third, fourth and fifth groups were labelled as extract groups and treated with methanolic extract of *Tamarix Aphylla* at a dose of 150, 300 and 450 mg/kg respectively. After 16hrs of dosing, stool production (total number of normal as well as wet faeces) was then monitored in all five groups for 16 hours after dosing the rats.

Table 4: Division of Animal models into groups (n =30)

S. No	Group	Type	Drug Administered
1.	Group-I	Negative control	Normal Saline (NaCl)
2.	Group-II	Positive Control	Senna (10mg/Kg)
3.	Group-III	Extract Group-I	150mg/Kg
4.	Group-IV	Extract Group-II	300mg/Kg
5.	Group-V	Extract Group-III	450mg/Kg

Statistical analysis: Data were shown as means ± standard deviation. One way analysis of variance (ANOVA 95% confidence interval) was applied for measuring the difference between test animal groups and the control groups. A *p*-value less than 0.05 was considered significant.

RESULTS

Lethal Dose (LD_{50})

The methanolic extract of *Tamarix aphylla* was found to be safe at 1500 mg/kg (**Table 5**). Furthermore, 66.66% deaths occur at 1800 mg/kg and 100% deaths occur at 2100 mg/kg, 2400 mg/kg 2700 mg/kg and 3000 mg/kg dose respectively, so to calculate the LD50 i.e., 50% death we repeat the dosing between 1500 and 1800 mg/kg. No deaths occur again at 1500 mg/kg while 33.33% deaths occur at 1600 mg/kg 50% deaths occur at 1650mg/kg. So, it was concluded that LD_{50} of *Tamarix aphylla* leaf extract is 1650mg/kg in mice.

Table 5: Calculating LD_{50} of *Tamarix aphylla* leaf Extract

Dose mg/kg (p.o.)	**Mortality after 24 hrs**	**survived after 24 hrs**	**Mortality % after 24 hrs**
250	0	6	0
500	0	6	0
750	0	6	0
1000	0	6	0
1250	0	6	0
1500	0	6	0
1600	2	4	33.33
1650	3	3	50
1800	4	2	66.66
2100	6	0	100
2400	6	0	100
2700	6	0	100
3000	6	0	100

Effect of *Tamarix aphylla* on Charcoal Meal Gastrointestinal Transit Time

The GI motility test results are given in **Table 6**. In a concentration-dependent manner, the methanolic extract of *Tamarix Aphylla* enhanced the peristalsis of the charcoal meal over the GIT. No significant effect was observed at the dose of 150 mg/kg (*p.o.*) of the methanolic extract of *Tamarix Aphylla*, but at a dose of 300 mg/kg (*p.o.*) of the methanolic extract produced a

substantial increase in movement of the charcoal meal compared to control group (normal saline, 5 mL/kg, *p.o*.) ($p < 0.05$). Neostigmine (1ug/kg, i.p.) gastrointestinal motility effect was greater than the maximum dose of the extract (300 mg/kg, *p.o*.) used.

Table 6. Effect of *Tamarix aphylla* and neostigmine on small intestinal transit in mice.

Treatment	Total length of intestine (cm)	Distance traveled by charcoal (cm)	% Intestinal Transit	*p*-value
Negative control (NS)	47.68 ±1.15	23.12±1.33	48.48±2.43	-
Positve control 1ug/kg	47.1±1.68	38.85±1.28	82.57±4.22	-
Extg1 150 mg/kg	48.08±1.49	28.12±1.88	58.44±2.82	**<0.05**
Extg2 300 mg/kg	46.92±2.17	34.70±2.17	74.07±5.46	**<0.05**

Effect of *Tamarix aphylla* on Weight of The Faeces Matter

Concentration-dependent increases in faecal output of rats were noticed at different doses of extract when it was compared to control groups. For the control group and extract group-1 (EXG-1) at 150 mg/kg dose, no substantial change was noticed. In the laxative test extract groups (EXG-2 and EXG-3), 300 and 450 mg/kg significantly increased the diarrhoeal faeces output. As compared to the negative control group increase of diarrhoeal faeces was conspicuously high. Senna the standard drug increases diarrhoeal faeces. The results are summarized in **Table 7.**

Table 7: Faeces Output in Experimental Animals

Treatment	NS	Senna	EXG-1	EXG-2	EXG-3
Groups	1.60	* 4.70	2.23	** 2.46	*** 2.66
SD	0.04	0.48	0.20	0.07	0.08
***p*-value**	-	-	**>0.05**	**<0.05**	**<0.05**

DISCUSSION

Different pharmacological interventions are being in practice for the treatment of constipation including herbal medicine practitioners. Gastrointestinal motility is related to the contraction and relaxation of smooth muscles of the GI tract. Because of this, the movement of the gastrointestinal contents is regulated. The peristaltic motility of GIT is under the control of both the hormonal and neuronal systems. In critically ill patients, the GIT motility disorders are very common which may require appropriate and prompt care. This reduces the chances of unwanted healthcare outcomes and patient's stay at the hospital. The clinical presentations of GI motility disorders may vary, depending upon the part of GIT involved and the pathophysiological causes. According to Rauch

et al, the large intestine transit time for wireless motility capsule was significantly higher than in healthy individuals i.e., 10 days vs 1.2 days (21). On the other hand, Mukai et al investigated the effects of the high-fat diet on the defecation period in mice. It was found that a diet rich in fats causes constipation in mice by decreasing colonic mucus secretion (22).

In the current study, the lethal dose (LD_{50}), prokinetic and the laxative effects of methanolic extract of *Tamarix aphylla* were determined. For this purpose, a series of experiments were conducted to find out the safe dose range. As per our results, 1650 mg/kg was calculated to be the estimated LD50 (mg/kg) for *Tamarix aphylla* methanolic extract in mice. Higher doses (>1650 mg/Kg) resulted in a subsequent increase in mortality rate. We also found that the extract of *Tamarix aphylla* had significant laxative properties. Upon oral administration of methanolic extract of *Tamarix aphylla* (150-300 mg/kg), there was a significant dose-dependent increase in the gastrointestinal motility as observed in the charcoal meal transit test. This showed its prokinetic activity. The results were compared with the standard laxative drug i.e., neostigmine, having prokinetic activity, which was found to be significant (p-value <0.05). Similarly, the percentage of intestinal transit length was also significantly increased by the methanolic extract of *Tamarix aphylla* when compared with standard drug i.e., neostigmine.

The laxative activity of *Tamarix aphylla* methanolic extract was also determined in our study. No significant difference in the increase in faecal counts was found between the negative control group and extract group-1 receiving normal saline and *Tamarix aphylla* methanolic extract at a dose of 15mg/Kg. however, at higher doses i.e., 300 mg/kg and 450 mg/kg, a substantial laxative effect on the faeces count was found. At these doses, extract groups of 300 and 450 mg/kg increased the diarrhoeal faeces output i.e., 2.46 ±0.07 (**p<0.05), and 2.66±0.08 (***p<0.05) respectively. When compared to the negative control group, the increase of diarrhoeal faeces was conspicuously high. Senna, the standard drug increases the diarrhoeal faeces 4.7±0.48 (*p<0.05), *Tamarix aphylla* (300 and 450mg/kg) showed a positive impact on wet faeces in rats.

CONCLUSION

This study concluded that *Tamarix aphylla* had a potential laxative effect. The findings of this study validate the use of the leaves of *Tamarix Aphylla* as a laxative in traditional medicines. Further studies may be conducted to find out the bioactive ingredients that are responsible for the laxative activity of the plant and also the exact mechanism of action involved in producing such effects in animal models.

REFERENCES

1. Barberio B, Judge C, Savarino EV, Ford AC. Global prevalence of functional constipation according to the Rome criteria: a systematic review and meta-analysis. The Lancet Gastroenterology & Hepatology. 2021.
2. Black CJ, Ford AC. Chronic idiopathic constipation in adults: epidemiology, pathophysiology, diagnosis and clinical management. Medical Journal of Australia. 2018;209(2):86-91.
3. Raza J, Jaffary A, Ahmed M, Mohiuddin K, Dastgir A. Lichtenstein repair; a community based teaching hospital experience. Rawal Med J. 2008;33:169-73.

4. Sobrado CW, Corrêa IJF, Pinto RA, Sobrado LF, Nahas SC, Cecconello I. Diagnosis and treatment of constipation: a clinical update based on the Rome IV criteria. Journal of Coloproctology (Rio de Janeiro). 2018;38:137-44.
5. Arco S, Saldaña E, Serra-Prat M, Palomera E, Ribas Y, Font S, et al. Functional constipation in older adults: Prevalence, clinical symptoms and subtypes, association with frailty, and impact on quality of life. Gerontology. 2021:1-10.
6. Sumner J. The natural history of medicinal plants: Timber press; 2000.
7. Prabhavathi N, Kowsalya B, Kumar SR, Sravani BJ, Sri GD, Sakila A, et al. Analgesic activity of different solvent extract of Operculina turpethum by using swiss albino mice. Asian J Pharm Clin Res. 2012;5(3):215-8.
8. Méité S, Bahi C, Yéo D, Datté JY, Djaman JA, N'guessan DJ. Laxative activities of Mareya micrantha (Benth.) Müll. Arg.(Euphorbiaceae) leaf aqueous extract in rats. BMC complementary and alternative medicine. 2010;10(1):7.
9. Majumder P, Paridhavi M. A Comprehensive Ethno-Phyto-Pharmacological Review on Novel Indian Medicinal Plants Used In Polyherbal Formulations. International Journal of Phytomedicine. 2013;5(4):394.
10. Degen L, Matzinger D, Merz M, Appel-Dingemanse S, Osborne S, Lüchinger S, et al. Tegaserod, a 5-HT4 receptor partial agonist, accelerates gastric emptying and gastrointestinal transit in healthy male subjects. Alimentary pharmacology & therapeutics. 2001;15(11):1745-51.
11. Jasiem TM, Nasser NM, AL-Bazaz HK. Tamarix aphylla L.: a review. Research Journal of Pharmacy and Technology. 2019;12(7):3219-22.
12. Mohammedi Z, Atik F. Impact of solvent extraction type on total polyphenols content and biological activity from Tamarix aphylla (L.) karst. 2011.
13. Marwat SK, Rehman F. Medicinal folk recipes used as traditional phytotherapies in district Dera Ismail Khan, KPK, Pakistan. Pak J Bot. 2011;43(2):1453-62.
14. Qadir MI, Abbas K, Hamayun R, Ali M. Analgesic, anti-inflammatory and anti-pyretic activities of aqueous ethanolic extract of Tamarix aphylla L.(Saltcedar) in mice. Pak J Pharm Sci. 2014;27(6):1985-8.
15. Zain ME, Awaad AS, Al-Outhman MR, El-Meligy RM. Antimicrobial activities of Saudi Arabian desert plants. Phytopharmacology. 2012;2(1):106-13.
16. Mohammedi Z, Atik F. Fungitoxic effect of natural extracts on mycelial growth, spore germination and aflatoxin B1 production of Aspergillus flavus. Australian Journal of Crop Science. 2013;7(3):293.
17. Ashour OM, Abdel-Naim AB, Abdallah HM, Nagy AA, Mohamadin AM, Abdel-Sattar EA. Evaluation of the potential cardioprotective activity of some Saudi plants against doxorubicin toxicity. Zeitschrift für Naturforschung C. 2012;67(5-6):297-307.
18. Sheikh DK, Dixit AK. Plants in the holy quran: a look. World J Pharm Pharm Sci. 2015;4:715-38.
19. Balamurugan R, Ignacimuthu S. Antidiabetic and hypolipidemic effect of methanol extract of Lippia nodiflora L. in streptozotocin induced diabetic rats. Asian Pacific journal of tropical biomedicine. 2011;1(1):S30-S6.
20. Croci T, Landi M, Emonds-Alt X, Fur G, Maffrand JP, Manara L. Role of tachykinins in castor oil diarrhoea in rats. British journal of pharmacology. 1997;121(3):375-80.
21. Rauch S, Krueger K, Turan A, You J, Roewer N, Sessler DI. Use of wireless motility capsule to determine gastric emptying and small intestinal transit times in critically ill trauma patients. Journal of critical care. 2012;27(5):534. e7-. e12.
22. Mukai R, Handa O, Naito Y, Takayama S, Suyama Y, Ushiroda C, et al. High-fat diet causes constipation in mice via decreasing colonic mucus. Digestive diseases and sciences. 2020;65(8):2246-53.