Original Article

Evaluation of Analgesic Activity of the Ethanolic Extract of Cuscuta Reflexa Stems in Albino Wistar Mice

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ABSTRACT

Objective: The goal of the current study is to assess the palliative / analgesic action of Cuscuta Reflexa stem extract in comparison with standard drug Morphine.

Study Design: A Experimental study

Place and Duration of Study: This study was conducted at the University of Karachi in Karachi, Pakistan, for nine months from February 2020 to November 2020.

Materials and Methods: The pain- relieving effect was assessed by using Tail Flick method to induce pain in the albino mice and the latency period was noted. Four groups of animals were made. Each group has seven albino mice, viz. control, standard drug and extract-treated with two different dosage assemblies.

Results: Cuscuta Reflexa contributes a dose-dependent pain-relieving effect using Tail Flick method in Albino mice.

Conclusion: The outcomes represent Cuscuta Reflexa extract has important pain-relieving constituents. These outcomes support the traditional use of Cuscuta Reflexa for the management of pain using morphine.

Key Words: Cuscuta Reflexa, pain, palliative/ analgesic, morphine, albino mice

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INTRODUCTION

Pain is an aggressive physical and expressive involvement connected with physical or probable soft tissue injury.[1] Pain is constantly an individual involvement that is subjective to unpredictable evaluations by means of natural, emotional, and public influences.[2]

Now-a-days most common drugs for the relief of pain includes NSAIDS (Non-Steroidal Anti-Inflammatory Drugs) and opioids especially Morphine as well as Aspirin.[3]

As far as the analgesic effect is concern these NSAIDS and Opioids are considered as the most potent analgesic and frequently used in case of chronic pain and Post-operative pain.[4] Morphine and heroin are used as leisure medications and require the capability to make opioid addiction.[5] Therefore the use of Opioids and NSAIDS are decreased due to addiction and adverse effects problems.[6]

Consequently, natural source of a drug is favorable in such cases because of minimal or no side effects.[7] Drugs obtained from natural origin possesses remarkable effects like antioxidant, anti-proliferative, anti-microbial, anti-hypertensive, anti-hyperlipidemic properties, anti-emetic etc.[8]

Cuscuta Reflexa is usually recognized as dodder, devil's hair and akash bel.[10] It is a member of the Convolvulaceae family. It's an amazing plant with a long history of therapeutic and medicinal value, found throughout the tropical and subtropical regions of the world.[11]

Cuscuta Reflexa contain lots of secondary metabolites includes terpenoids, alkaloids, saponins, flavonoids and steroids.[12] It has traditionally been used to treat chronic ulcers, fever, chest pain, itching, jaundice, nephrotic issues, and migraines.[13,14] It is used to treat tumours and fevers and has anti-diabetic, anti-convulsant, and anti-inflammatory properties.[15]

MATERIALS AND METHODS

Preparation of Extract: Cuscuta Reflexa stem was shade-dried, crushed, and sifted through a (40 mesh)

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sieve before being stored in an amber-colored glass bottle. 500 grammes of Cuscuta Reflexa crushed stem was dispersed in 1000ml of Ethanol and left for 30 days. The solution was then examined using a Whatmann strainer, and the solvent was removed using a Rotatory evaporator. The ethanolic extract was stored at 4 °C in an amber-colored glass bottle for future research. [4]

Experimental Animals: Albino male and female Wistar mice (20-25g) were obtained for the study from the Animal House, Department of Pharmacognosy, University of Karachi. The animals were kept in typical laboratory settings at a constant temperature and humidity levels of 70-80%. An animal was accustomed to the routine examination laboratory conditions of 12 hours of sunlight and 12 hours of darkness and was fed in accordance with rat food. Mortals must fast for 12 hours prior to dosing.

Grouping of Experimental Animals: The animals are split into four distinct groups, each with ten animals.

Group A: Group of controls (0.5ml of normal saline)
Group B: Morphine (10mg/kg) regular dosage
Group C: Cuscuta Reflexa (50mg/kg) dosage
Group D: Cuscuta Reflexa (100mg/kg) dosage

Analgesic activity can be evaluated by using Tail Flick method. This technique is used to determine the time of the tail flick reflex after heat induction. The thermal source is aimed at the distal part of the tail, approximately 2-3cm above the tip. The tail of mice is immersed in hot water at 51°C with a constantly measured temperature, and the likelihood of flicking the tail is assessed at 0 min. Administer the analgesic medication and wait for it to take effect (20-30 minutes). Repeat the steps, taking 5-7 readings at 30-minute intervals.

For the purpose of functional group activity, the FT-IR (Fourier transformer infrared) spectroscopic technique was used. This study made use of a Bruker FT-IR spectrophotometer (20).1-2 plunges of 0.1 ml Cuscuta Reflexa extract went through processing with a wave number range of 3500-500 cm1. Utilising adopted spectra, the utmost peak corresponding to functional groups has been recorded and equated with library records. Interval of 30 minutes.

Different Phytoconstituets are evaluated by using standard tests because Cuscuta Reflexa is rich in Phytocostituets like alkaloids, tannins , saponinns , glycosides etcc are present.[16]

RESULTS

The results of the analgesic activity of the ethanolic extract Cuscuta Reflexa are shown in Table 1. The ethanol extracts of stem of Cuscuta Reflexa is non-toxic and has a fatality rate of up to 2 g/kg in Swiss albino mice. Throughout the 180 minute observation, albino mice treated with normal saline (the negative control) didn't exhibit any appreciable differences in their reaction times to tail flicks. The increase in reaction time for morphine sulphate varied significantly between time points when compared to the baseline values within the same treatment groups. There was a significant improvement in tail flick method reaction time after using two different doses of Cuscuta Reflexa stem extract. Morphine sulphate and Cuscuta Reflexa extract-treated animals had significantly longer reaction times than saline-treated animals. The morphine reaction time was 2.48 seconds at 120 minutes, while the saline reaction time was 0.92 seconds and the Cuscuta Reflexa 1.29 sec (50mg/kg) and Cuscuta Reflexa 1.45 sec (100mg/kg) groups, respectively.

Table No. 1: Analgesic activity of Ethanolic extracts of Cuscuta Reflexa by using tail flick method

Group	Treatment	Dose (mg/kg per-oral)	Reaction Time (in Seconds) (mean±SEM)						
			0 min	30 min	60 min	90 min	120 Min	150 Min	180 Min
Control	Distilled water	0.5	0.89± 0.01	0.90± 0.01	0.91± 0.01	0.91± 0.01	0.92± 0.01	0.90± 0.00	0.90± 0.00
Standard	Morphine	10	0.86± 0.02	1.17± 0.01***	1.79± 0.01**	2.26± 0.03*	2.48± 0.02*	2.35± 0.02*	2.08± 0.04*
Test 1:	CR	50	0.90± 0.01	1.00± 0.01**	1.12± 0.01**	1.25± 0.02*	1.29± 0.02*	1.20± 0.00**	1.05± 0.01**
Test 2:	CR	100	0.90± 0.02	1.05± 0.01**	1.24± 0.00**	1.42± 0.02*	1.45± 0.02*	1.30± 0.02*	1.05± 0.01**

(N=7) each data suggest Mean±SEM.

One-way Analysis of Variance (ANOVA) followed by post hoc tukey's multiple comparison test is applied for statistical analysis.

*Significant at P<0.05, ** Significant at P<0.01 vs. Normal control

The screening of Phytoconstituents present in the stem part of Cuscuta Reflexa was carried out by adopting the

standard test and the result which was obtained was mentioned in Table 2.

Table No. 2: List of Phtytoconstituents in Cuscuta Reflexa

S. #	Phytoconstituents Present	Cuscuta Reflexa Stem
1	Alkaloid	+
2	Anthraquinone	+
3	Carbohydrate	-
4	Flavanoid	+
5	Phenol	+
6	Tannins	+
7	Saponins	+
8	Glycosides	+
9	Quinones	-
10	Terpenoids	+
11	Protein	+
12	Phytosterols	+
13	Fixed oil	+

Note: + (positive result), - (negative result)

Cuscuta Reflexa's functional grouping was identified via FT-IR (Fourier transformation infrared spectroscopy).

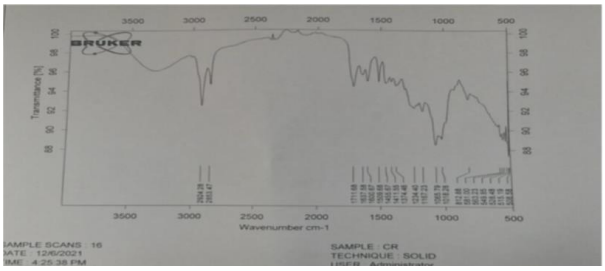

Figure No. 1: FT-IR spectrum of Cuscuta Reflexa

With the aid of a mortar made of agate and an FT-IR spectrometer operating in the area of 3500-500cm-1, Cuscuta Reflexa stem ethanol extract has been crushed into a finely ground powder.

Table No. 3: Ft-Ir Absorption Band Assignments of Cuscuta Reflexa

Peak's wavelength (cm-1)	Possible functional groups
1167	C-O
1234	C-N
1374	C-H-(CH3)
1411	C-H
1600	C=C
2924	O-H
2853	CH2 ,CH3

The wavelength cm-1 indicate the presence of specific group in the sample (Cuscuta Reflexa)

The strongest peak of alkane was observed in the Cuscuta Reflexa sample at 2853cm-1. The presence of polyphenolic compounds is indicated by the O-H peak stretching symmetrically at 2924 cm-1, and by the broad structural peak of conjugated alkenes at 1600 cm-1. The occurrence of an amine group is revealed by the topmost C-O at 1234 cm-1, whereas the occurrence of anhydride, ether, carboxylic acid, and alcohol is revealed by the C-O at 1167 cm-1.

DISCUSSION

Centrally acting analgesics work by aggregate the pain threshold and adjusting the physiological response to pain. Peripherally acting analgesics, in contrast hand, work by stopping impulse invention at the chemoreceptor site of pain.

In the current research, analgesic activity was assessed using pain-state models and stimuli such as the tail-flick method. In the tail-flick model, the ethanolic extract from the flower of Cuscuta Reflexa showed considerable analgesic activity by prolonging the reaction time of albino mice compared to control (saline treated mice) at all points of time.

Morphine sulphate, a moderate to severe analgesic[18], was used as the standard drug. Compared to the control, morphine had the greatest anti-nociception affect throughout all observation times, followed by the extract. The tail-flick technique is based on the discovery that morphine-like compounds specifically lengthen the response time of the usual tail-withdrawal effect in mice. This technique is also useful for differentiating between central opioid-like analgesics and peripheral analgesics.

Mushtaq et al. discovered that the ethanolic extract of Cuscuta Reflexa stem created dose dependent and substantial analgesic and anti-inflammatory activity as compared to standard drug diclofenac sodium.[18] Moreover, using various animal models, an Indian study established the anti-nociceptive and anti-inflammatory activity of a petroleum ether extract of Cuscuta Reflexa leaves.[19] Several trainings have been conducted on Cuscuta Reflexa, but to our knowledge, none of them have assessed the analgesic potential of the stem extract.

CONCLUSION

As a final point, the ethanolic extract of Cuscuta Reflexa confirmed analgesic activity, surely donating to the traditional use of medicinal plant in pain assistance. Moreover, investigation is required to recognize the active compounds or constituent in this extract and to know the mechanisms behind its pain-relieving goods.

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Author's Contribution:

Concept & Design of Study:	Qurat ul Ain Sameed
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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