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Original Article

Phytochemical Standardization and Anti-Anxiety (*Izterab-e-Nafsani*) study of *Aftimoon Hindi* (*Cuscuta reflexa* Roxb.) on An Animal Model

Irfat Ara[1], Mohd Afsahul Kalam[2], Mudasir Maqbool[3*], Mehrukh Zehravi[4]

[1]Research officer Unani Scientist-4, Regional Research Institute of Unani Medicine, University of Kashmir, Srinagar, Jammu and Kashmir, India, [2]Research officer Unani Scientist-1, Regional Research Institute of Unani Medicine, University of Kashmir , Srinagar, Jammu and Kashmir, India, [3]Research Scholar, Department of Pharmaceutical Sciences, University Of Kashmir, Srinagar, Jammu and Kashmir, India, [4]Lecturer, Department of Clinical Pharmacy Girls Section, Prince Sattam Bin Abdul Aziz University Alkharj, Saudia Arabia*

ABSTRACT

Background: *Cuscuta reflexa* Roxb is a member of the *Cuscutaceae* family, and in Unani medicine, it is known as *Aftimoon*. It is a parasitic plant that can be found growing abundantly on various host plants in India up to 3000 metres in altitude during the rainy season. Unani physicians have been using it for years to cure a variety of illnesses, including psychiatric illnesses like melancholia, schizophrenia, and epilepsy. It has been used to cure hepatitis, palpitations, and skin disorders, among other things. **Objective of the study**: To evaluate anti-anxiety effect of *Cuscuta reflexa* Roxb in Swiss Albino mice of either sex. **Materials and Methods**: A total of 24 Swiss Albino mice weighing 25-35 g were used in this study. Animals were chosen at random and held in their cages for at least 7 days in a standard setting. Group A was given regular saline as a vehicle, Group B was given a hydro alcoholic extract of the lower dose of the test drug, Group C was given a hydro alcoholic extract of the higher dose of the test drug, and Group D was given the standard drug Diazepam 5 mg/kg orally. *Aftimoon* as hydro alcoholic extract (200 mg/kg and 400 mg/kg body wt.) was given in single and double doses and observed for 7 days. **Results**: For each parameter in each category, mean and standard deviations were computed. For multiple group comparisons, a one-way ANOVA was used, followed by Turkey's post hoc test. ($p<0.05$) was used as the significance standard. **Conclusion**: These results advocate that the *Aftimoon* as double dose (400 mg/kg body wt.) revealed anti-anxiety effect similar to standard drug.

Keywords *Aftimoon Hindi,* Anxiety Disorder*, Izterab-e- Nafsani,* Unani Medicine

1. INTRODUCTION

Anxiety disorder is a mental health condition characterised by excessive worry, anxiety, or apprehension that interferes with daily activities (Robichaud and Dugas, 2019; Reddy, 2010). Panic disorder with or without agoraphobia, generalised anxiety disorder, social anxiety disorder, particular phobias, and separation anxiety disorder are among the most common psychological illnesses (Bandelow and Michaelis, 2015). Anxiety disorders affect up to 20% of adult population per year. The signs of generalised anxiety disorder include fear, worry, and a constant sense of frustration. GAD is characterised by excessive, irrational anxiety about everyday activities *(Löwe et al.,* 2008). Symptoms resembling GAD are classified under various mental disorders in the Unani System of Medicine, particularly in the primary stage of melancholia, despite the fact that there is no specific diagnosis of anxiety disorders (Khan, 2011; Ara *et al.,* n.d.; Ansari *et al.,* 2020; Hkm, 2006). Generalized Anxiety Disorder (GAD) is literally translated as *Izterabe Nafsani Umoomi* since the phrase "*Izterab*" is used for anxiety in Arabic and Unani documents and the word "*Nafsani*" is applied to *Izterab* to specify its psychological state. *Quwwat-e-Mufakkirah* (faculty of thoughts), which is part of *Quwwat-e-Mudrika Batina* (internal perceptive faculties) of *Quwwat-e-Nafsaniyah* (Unani System of Medicine), regulates anxiety (Psychic faculty). Most of these brain or psychic faculties, particularly *Sauda* and *Balgham*, are susceptible to these humours and are affected by some psychological or pathological abnormality, according to Unani Medicine's Humoral Concept. The primary aim of nervous system pharmacology, like anxiety, is to alleviate these morbid humours. The herb *aftimoon Hindi* (*Cuscuta reflexa* Roxb) is used in Unani medicine to treat nervous system disorders (Nadkarni, 1996; Sisay and Gashaw, 2017; Rajaram *et al.,* n.d.; Miraj and Kiani, 2016; Ara *et al.,* n.d.; Akbar, 2020).

Aftimoon (*Cuscuta reflexa* Roxb.) is a parasitic plant used in the Unani system of medicine to treat vitiligo, pityriasis, depression, epilepsy, and other mental diseases. It is available as a *mufrad* (single drug) in powder, decoction, mixture, and *murakkab* (compound formulations). The plant belongs to the

*Correspondence: Mudasir Maqbool
E-mail: bhatmudasir.scholar@kashmiruniversity.net
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Cuscuta genus and the *Cuscutaceae* family. This parasite plant grows up to thousands of feet on different host plants in India and is found abundantly throughout the rainy season. It is yellowish in hue, with a slightly swirled stem which is frequently termed Dodder in English on other plants and trees. Unani physicians have been employing this plant for numerous diseases relating to the body's humour problem from time immemorial. A number of biologically active substances such as amarbelin, cuscutin, cuscutalin, etc. have been isolated and important and pharmacological actions such as the use of *aftimony* (*Cuscuta reflexa* Roxb) have been reported in several studies, including anti-cancerous, anti-tumoral, anti-inflammatory, antibacterial, etc. *Cuscuta reflexa* is a potential medication, even if it is a plant parasite, but can be used as an alternative medicine source in a number of conditions. In the Unani classical texts, extensive details about uses and pharmacological activities have been mentioned but evidence needs to be generated through the tests and yet the afflicted masses need a lot of healing from natural sources which leads to and strengthens research into the Unani system of medicine (Thomas *et al.,* 2015; Patel *et al.,* 2012; Bandelow *et al.,* 2012).

Keeping in the view, the whole epidemiology, globally and Kashmir in particular the anxiety disorder was chosen as study disease for preclinical trial. Reviewing the Unani literature anxiety disorders fall in the category of *Fikr* or *Izterabe Nafsani*. There are number of herbal drug mentioned in Unani literature since a long time for the management of anxiety disorder like; *Kishneez (Coriandrum sativum), Barhami (Bacopa moneri), Sankhaholi (Evolvulus alsinoides), Halela Siyah (Terminalia chebuala), Halela Zard (Terminalia chebula), Kahu (Lactuca sativa), Neelofar (Lotus nymphea), Gule Surkh(Rosa damascene), Aftimoon (Cuscuta reflexa)* and some compound drugs like *Majoon Najah, Sharbat Ahmad Shahi, Qurs Dawaus Shifa, Roghan Kaddu, Roghan Kahu etc* (Hkm, 2006; Hkm, 1999; Majeedi, 1999; Ara *et al.,* 2020; Ara *et al.,* n.d.). Out of which the herb *Cuscuta reflexa* Roxb also known as "*Aftimoon Hindi*" and "*Akashbel*" in Unani medicine, is a short size herb having potent anxiolytic activity, Hence a preclinical animal study was envised to explore the anxiolytic effect of the drug to validate the claim and enhance the way for its clinical study.

MATERIALS AND METHODS

The current research, titled "Phytochemical Standardization and Anti-Anxiety (*Izterab-e-Nafsani*) Analysis of *Aftimoon Hindi* (*Cuscuta reflexa* Roxb.) On Animal Model," was conducted at the Regional Research Institute of Unani Medicine (RRIUM) and the University of Kashmir's *Ilmul Advia* department. The protocol was submitted for ethical approval before the analysis began. The protocol (IAEC-Approval) KU/2017/16 was approved by the Institutional Ethical Committee. Swiss Albino Mice of either sex were used in the experiment. *Cuscuta reflexa* Roxb was obtained from a reliable source. Botanists were able to identify the compound. The data came from experiments conducted at RRIUM Srinagar's drug standardization research unit (DSRU) and the University of Kashmir's pharmacology laboratory department of Pharmaceutical Sciences.

Physiochemical Evaluation of Drug:
Physiochemical evaluation of plant drug sample was done as per WHO guidelines for herbal drugs which includes; morphological characters, microscopic characters, loss on drying, acid insoluble ash, total ash, extractive value, ash value, total ash, foreign matter analysis, water soluble ash. Physiochemical screening of plant extracts includes foaming index, determination of pH, determination of flavanoid contents, phenolic contents.

Test Drug (Dose and route of administration)
Aftimoon Hindi was procured from authentic sources and dried in shade and made powdered by electric grinder. The hydro-alcoholic extract were obtained by using soxhlets apparatus. The human therapeutic dose of this drug has been mentioned in Unani literature has 7 gm. The dose for mice is calculated by conversion factor 12 and found to be 200 mg/Kg for *aftimoon* respectively. A second dose was also taken that is just double of first dose and found to be 400 mg. The dose of the extract was measured with reference to the dose of crude drug after the yield percentage of extract was obtained.

Experimental design
Swiss Albino mice of either sex weighing 25-35 g were used in the experiment. The animals were housed in a standard setting with unlimited access to food and water. Swiss Albino mice weighing 20-25 g were divided into four classes, each with six animals.
Group A: Control, administered normal saline as vehicle orally
Group B: Received Hydro alcoholic extract 200 mg/Kg
Group C: Received Hydro alcoholic extract 400 mg/Kg
Group D: Received standard drug Diazepam 0.5mg/kg orally.

Instrument: Elevated plus-maze (EPM)
The EPM apparatus consisted of two open arms (35cm x 5cm) and two closed arms (30cm x 5cm x 15cm) that extended from a standard central platform (5cm x 5cm) elevated to a height of 50 cm above floor level. Mice were given oral doses of plant extracts 30 minutes before being put on the EPM. The number of entries and time spent in the open and closed arms were recorded during a five-minute test period. The percentage of open arm entries for each species was calculated (100 x open/total entries). The following was used to calculate the percentage of time spent in the open arm:

$$\% = \frac{\text{Number of seconds spent in the open arm} \times 100}{\text{300 total sec (5 min observation periods)}}$$

Light and dark test
The unit consisted of a rectangular box (45x27x27) with a 7.5x7.5 cm opening in the wall dividing it into two compartments. One compartment was given a roof and was given a black paint job. The other compartment lacked a roof and was brightly illuminated by a 60 W bulb fixed above it. Mice were given oral doses of various plant extracts and then placed in the light compartment's centre for 5 minutes after a 30-minute observation period. The amount of time spent in the open compartment (white/light) was recorded. The time spent in the light compartment was calculated using the following formula:

$$\% = \frac{\text{Number of seconds spent in the light compartment} \times 100}{\text{300 total sec (5 min observation periods)}}$$

Open Field Test
The OF test is a 400x400x300 mm arena with thin black stripes drawn across the floor and divided into 16 quadratic blocks. It tests locomotion, exploration, and anxiety all at the same time. After each mouse was placed in the centre of the arena, an observer monitored the mouse's spontaneous ambulatory locomotion for 5 minutes. During this time, the number of squares crossed and rearing was also counted.

Statistical Analysis
For each parameter in each category, mean and standard deviations were measured. The data was analysed statistically using one-way ANOVA followed by the Dunnet Test.

RESULTS

Pharmcognostic evaluation of *Cuscuta reflexa*

Morphological Characters
Color: Brown
Odor: Characteristic
Taste: Slightly bitter
Size: Long Branched

Physiochemical Constants

Ash values
The total ash, acid insoluble and water soluble ash of *Cuscuta reflexa* was found to be 7.62, 0.91 and 4.61 % respectively.

Extractive Values

Table 1. Cold extractive values of *Cuscuta reflexa*

Solvents Used	Wt. of drug (g)	% yield (w/w)
Petroleum ether	5	1.38
Chloroform	5	2.84
Ethyl acetate	5	3.56
Methanol	5	7.24
Aqueous	5	6.02

Table 2. Hot extractive values of *Cuscuta reflexa*

Solvents Used	Wt. of drug (g)	% yield (w/w)
Petroleum ether	5	4.32
Chloroform	5	2.92
Ethyl acetate	5	1.90
Methanol	5	7.12
Aqueous	5	5.60

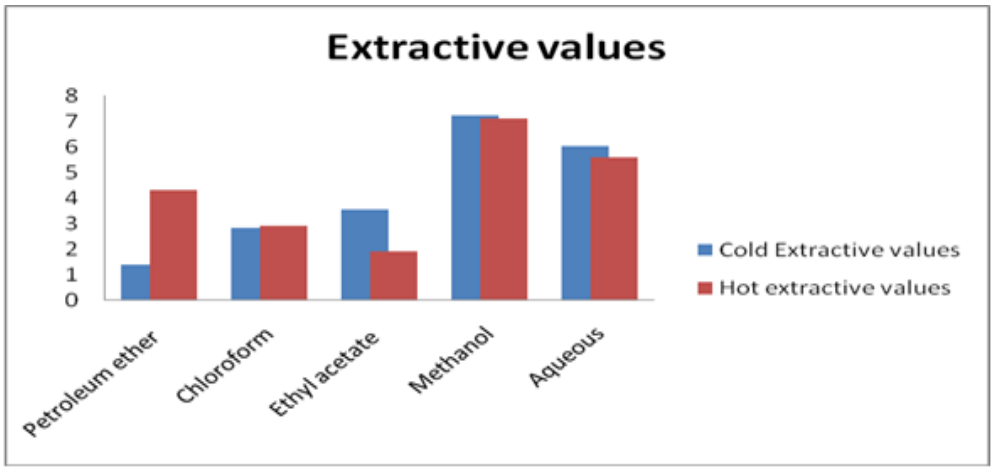


Fig. 1 Graphical representation of cold and hot extractive values of whole plant of *Cuscuta reflexa*

Foreign organic matter
The foreign organic matter of whole plant of *Cuscuta reflexa* was found to be 0.018%.

Loss on drying
The percentage LOD of whole plant of *Cuscuta reflexa* was found to be 6.89 %.

pH values
The pH of 1 and 10 % solution of whole plant of *Cuscuta reflexa* was found out to be 7.70 and 6.20 respectively.

Preliminary Phytochemical Screening

Table showing presence (+) and absence (-) of phytoconstituents of hydroalcoholic extract of *Cuscuta reflexa*

Table A. Preliminary Phytochemical Screening

Phytoconstituents	Tests	Result
Carbohydrates	Molish's test	+
	Fehling's test	+
	Benedict's test	+
Alkaloids	Dragendroff's test	+
	Mayers test	+
	Hagers test	+
	Wagners test	+
Saponins	Foam test	+
Glycosides	Keller killani test	-
	Borntrager's test	-
Flavonoids	Shinoda test	+
	Lead acetate test	+
Steroids	Salkowaski test	+
	Burchad's test	+

Amino acids & Proteins	Ninhydrin test Millon's test	+ +
Tannins	Ferric chloride test	+

Total Phenolic Content

Using the *Folin Ciocalteu* reagent, the total phenolic content was calculated and expressed in mg/gm of GAE. The hydroalcholic extract of the aerial portion of *Cuscuta reflexa* had a total phenolic content of 166.95 mg/g gallic acid equivalent.

Total Flavanoid Content

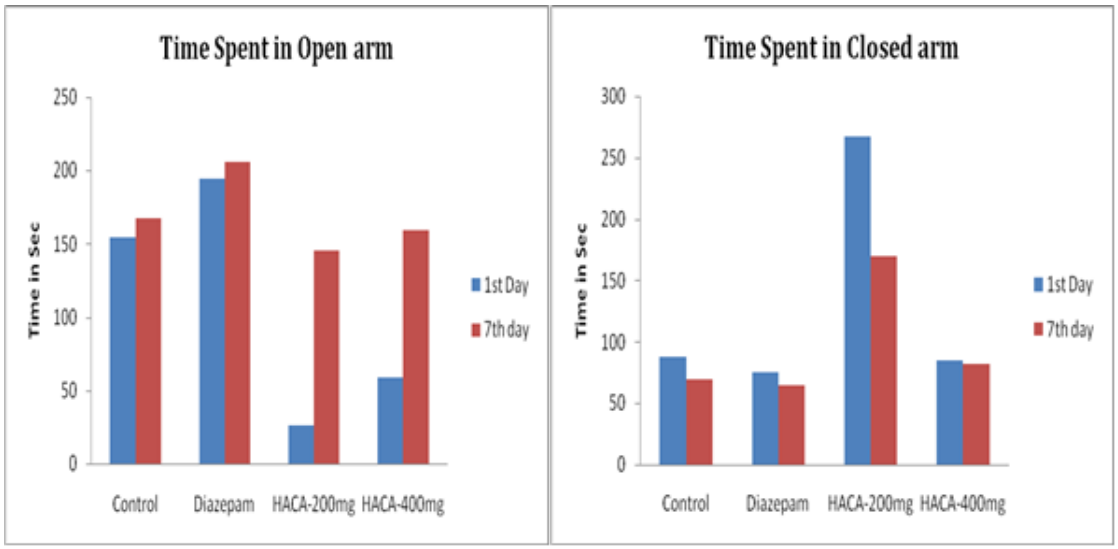
The Total Flavanoid content was expressed in mg/gm of Rutin. The total Flavanoid content of the hydroalcholic extract of aerial part of *Cuscuta reflexa* was 78.32 mg/g of Rutin

Table B. Anxiety related behavior using Elevated Plus-Maze model (EPM)

Groups	Days	Time Spent (Sec)		Number of Entries	
		Open arm	Closed arm	Open arm	Closed arm
Control	1st	154.33 ± 0.65	87.66 ±5.175	4.295±0.17	8.067±1.20
	7th	167.34±1.27	69.66±2.27	3.828±0.24	8.66±0.666
Diazepam(0.5mg/Kg)	1st	194.77±1.84**	74.90±3.13**	8.548±0.19**	5.33±1.476*
	7th	205.593±5.43**	64.16±1.18***	4.408±0.15*	6.197±0.95*
HACA (200mg/Kg)	1st	26.16±14.76 ns	267.16±4.2 ns	1.33±3.10 ns	2.66±2.21 ns
	7th	58.83±5.90 ns	169.33±3.36 ns	3.34±5.28 ns	4.17±2.24 ns
HACA (400mg/Kg)	1st	145.21±2.68 *	85.12±3.10**	6.21±2.33**	2.35±1.18**
	7th	159.23±3.42**	82.45±4.28**	4.25±2.48*	4.46±2.24**

Results are expressed as mean ± SEM (n=6).

*p<0.05, **p<0.01, ***p<0.001 compared with control data was statistically analyzed by ANOVA followed by Dunnet test (HACA- Hydro alcoholic extract of *Cuscuta reflexa.*



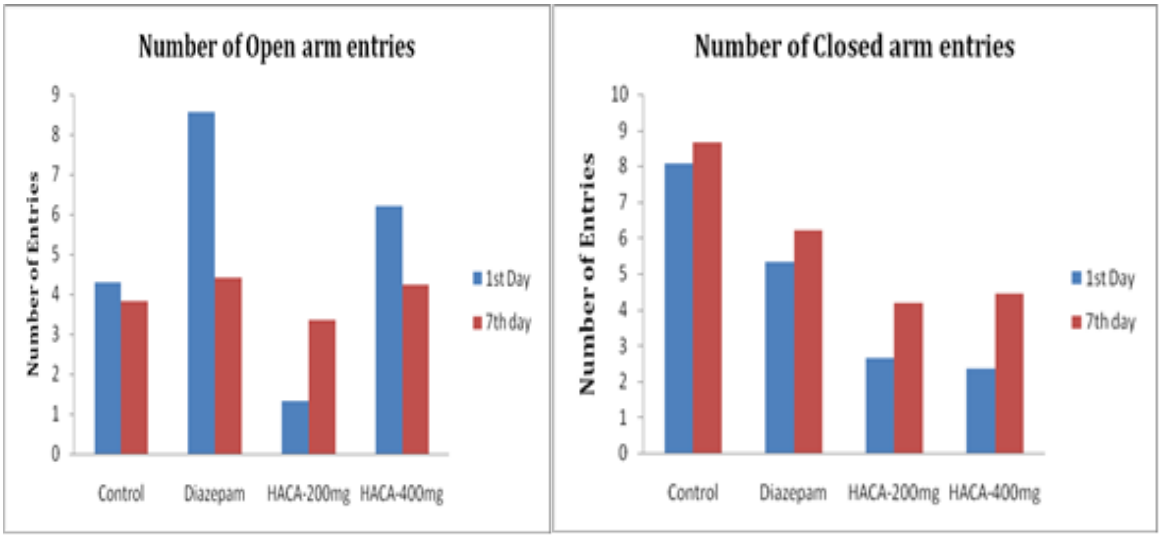
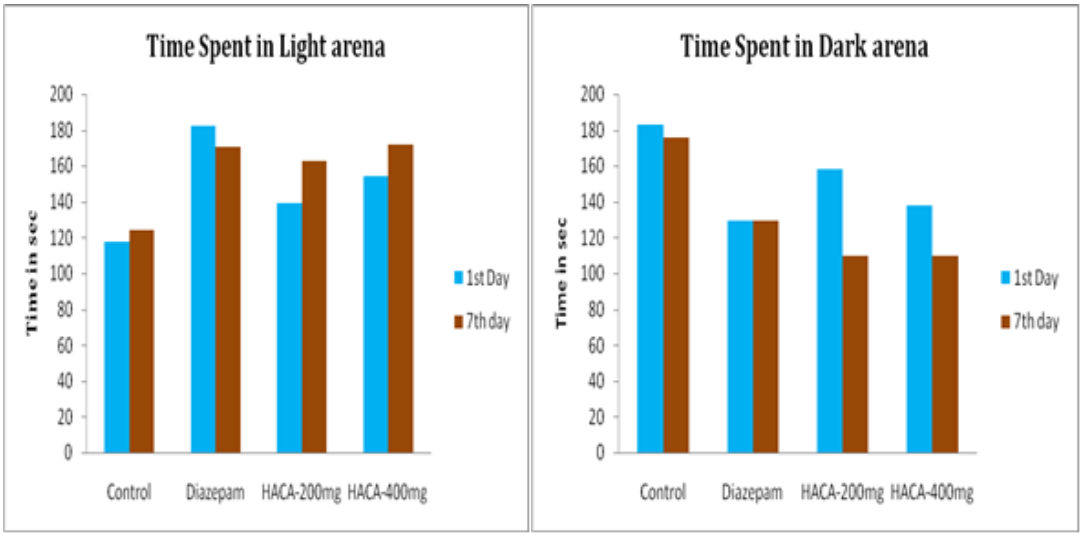


Fig. 2 Graphical illustration of influence of extract of *Cuscuta reflexa* on time spent in open/Closed arm in EPM model.

Table C. Light and Dark Test

Groups	Days	Time Spent (Sec)		Number of Entries	
		Light arena	Dark arena	Light arena	Dark arena
Control	1st	117.51±0.56	182.69±1.27	10.818±0.096	11.66±0.68
	7th	124.33±0.76	175.51±1.21	8.66±0.052	9.33±0.712
Diazepam(0.5mg/Kg)	1st	182.33±4.33**	129.66±1.16**	10.10±0.164**	10±0.11**
	7th	170.66±3.75**	129.41±0.53**	9.88±0.4**	10.6±0.137**
HACA (200mg/Kg)	1st	139±3.68**	158±4.52**	9±2.96**	7±3.42*
	7th	163±2.55**	110±7.24**	8±2.25**	7±3.46**
HACA (400mg/Kg)	1st	154±5.45**	138±5.22**	6±2.12**	8±4.21**
	7th	172±6.01**	110±7.25**	9±3.65**	6±3.22**



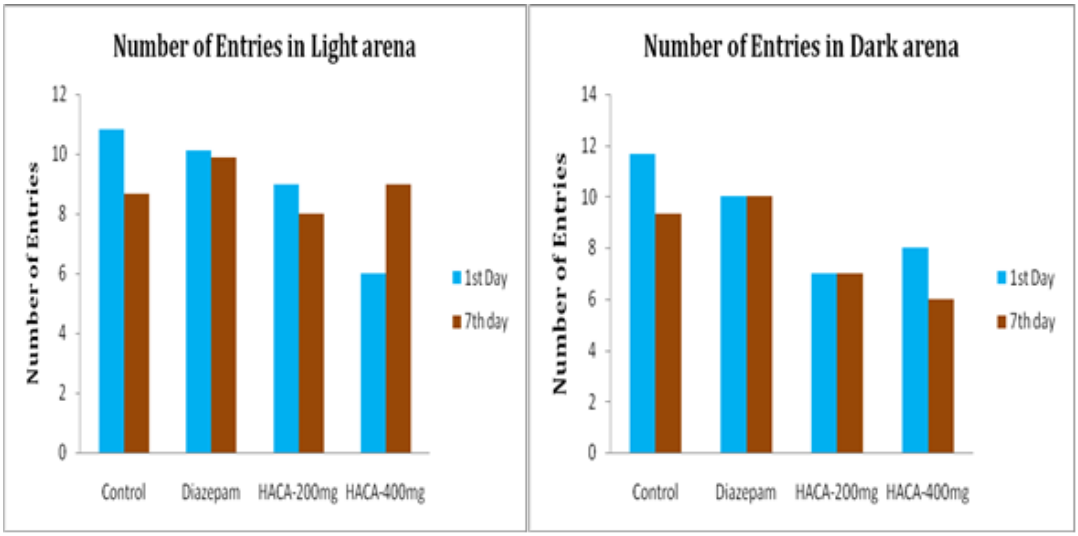


Fig. 3 Graphical illustration of influence of extract of *Cuscuta reflexa* on time spent in Light and dark arena model

Table D. Open Field Test

Items	Control	HACA	HACA	Standard Control (DZ)
Dose of Drugs (mg/Kg)	NS 1 ml	200 mg	400 mg	0.5 mg
Number of Squares Crossed	88.33±2.94	53.00±1.42	44.67±1.78	36.00±2.54
% Inhibition of Squares Crossing	2.3	40.0	49.3	59.24

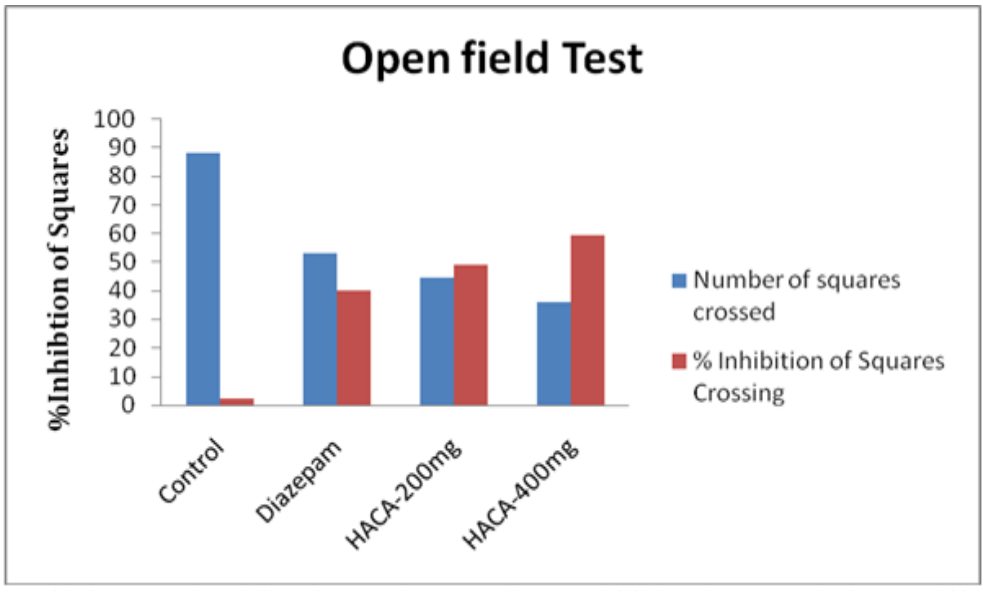


Fig. 4 Graphical representation of effect of extract of *Cuscuta reflexa* on % inhibition of square crossing in Open Field Model.

DISCUSSION

Orthodox healers have long used herbal remedies to cure anxiety disorders and provide relief, with no recorded side effects. The medicinal plant *Cuscuta reflexa* was chosen for anti-anxiety studies based on personal interactions with some of the traditional healers and locals in the collection areas, who say that the plant is used to reduce anxiety symptoms in animals. The present study entitled as "Phytochemical Standardization and Anti-Anxiety (*Izterab-e-Nafsani*) study of *Aftimoon Hindi* (*Cuscuta reflexa* Roxb.) On Animal Model" has been carried out at the department of *Ilmul Advia* in Regional Research Institute of Unani Medicine (RRIUM), and University of Kashmir-Srinagar. The aerial parts of the plants were studied for their pharmacognostic standardization and anxiolytic activity.

Pharmacognostic standardization and preliminary phytochemical analysis

The current study demonstrates numerous Pharmacognostic standardisation findings of the aerial parts of *Cuscuta reflexa*,

with the aim of potential investigational references and plant identification.

- The macroscopic analysis of drugs refers to the assessment of drugs based on colour, odour, taste, scale, shape, and other special characteristics such as touch and texture. It is a method of qualitative assessment focused on the examination of entire drug morphological and sensory profiles. Conclusions taken from studies arising from impressions on sense organs are referred to as organoleptic assessment (Kokate, Purohit and Gokhale 2005; Veeresham *et al.,* 2003). For the aerial parts of *Cuscuta reflexa*, all of these parameters were reported. These will aid in the initial detection of the crude drugs being investigated.
- Regulated incineration of crude drugs produces an ash residue made up of inorganic materials (metallic salts and silica). This parameter has a wide range of values, making it an important factor to consider when analysing crude drugs. The ash value immediately detects more direct pollutants like sand or earth. Inorganic variables in crude drugs, such as calcium oxalate, silica, and carbonate content, may also affect "total ash" values. The acid-insoluble ash value is determined after removing certain variables with acid (as they are soluble in hydrochloric acid) (Mukherjee, Kontz and Reinhardt, 2002). *Cuscuta reflexa*'s total ash, acid insoluble ash, and water soluble ash were found to be 7.62, 0.91, and 4.61 percent, respectively.
- Different extractive values determine the amount of active constituents in a specified amount of medicinal plant material when extracted with solvents. It's used to evaluate crude drugs that are difficult to test using other approaches. The amount of extractive drug yield to a given solvent is often used to estimate the amount of a particular constituent or group of related constituents in a drug. In certain cases, the amount of drug soluble in a given solvent may be used to determine its purity. The extraction solvent should be capable of dissolving large quantities of the target substances (Mukherjee *et al.,* 2002).
- The pH value, which defines the basic nature of the constituents, i.e. acidity or basicity, gives a small hint as to the form of constituents present. The basic range constituents are found in the *Cuscuta reflexa.*

The identity of the phyto-constituents present was determined through qualitative chemical tests. In phytochemical experiments, the hydroalcholic extract of *Cuscuta reflexa* was subjected to a variety of chemical tests in order to determine the identity of the constituents present. Chemical tests on the extract revealed the existence of a variety of essential constituents.

- The extracts of *Cuscuta reflexa* on hydroalcholic extract showed the presence of phytosterols, saponins, phenolics components, alkaloids, glycosides, tannins, terpenes and flavonoids.
- The Phenolic and flavonoid contents of aerial part of *Cuscuta reflexa* were found out to be as 166.95 mg /g of Gallic acid equivalent and 78.32 mg/g of Rutin respectively.

Anti-anxiety Studies

Mice of either sex were picked at random and divided into six groups for anti-anxiety testing. The elevated plus maze, light and dark arena, and open field test were used on the first and seventh days of the study to explore anti-anxiety effects. The number of entries and the amount of time spent in each arm were both recorded. The results were compared to those of a control group of healthy people. The current study shows that hydroalcholic extract of aerial sections of *Cuscuta reflexa* has antianxiety effects by demonstrating a strong anxiolytic effect using behavioural criteria such as EPM, light and dark, and open field tests. The EPM model of animal anxiety is now one of the most commonly used. EPM is focused on rodents' innate aversion to open spaces and exploits the tension between aversion and exploration. In the EPM, neophobia, discovery, and approach/avoidance conflict tend to be present in the elicited behaviour profiles (Bourin *et al.,* 2007). It's known as the "approach avoidance conflict model" because it takes advantage of a rodent's inherent opportunity to discover new environments (approach) and their intrinsic aversion to extremely hazardous open spaces (aversion) (avoidance) (Lach *et al.,* 2018). The most representative measures of anxiolytic behaviour are increases in open arm parameters. The total arm entries tend to represent shifts in anxiety or general behaviour, and time spent on the central platform appears to be linked to decision making and/or risk assessment (Blanco *et al.,* 2009).

First day study: Open arm activity in EPM is sensitive to the anxiolytic effect of diazepam. Diazepam as expected significantly increased the open arm entries (8.548± 0.19**) and the time spent in open arms (194.77± 1.84**sec) as compared to the control group (4.295± 0.17) and (71.66± 5.17sec). A decrease in the time spent in open arm compared to control group (129.33±0.65) indicating anxiolytic activity of diazepam.

In the same order as HACA, the hydroalcholic extract of aerial sections of *Cuscuta reflexa* increased open arm entries and time spent in open arm relative to the control group (400mg/Kg) (145.21±2.68 *) > HACA (200mg/Kg) (26.16±14.76 ns).

7th day study: After chronic care, each group was assessed on the seventh day of the study. The diazepam community showed an improvement in open arm exploration as compared to the first day 182.087±1.54** and hydroalcholic extract of *Cuscuta reflexa* (159.23±3.42**) compared with the control group.

The light and dark arena, which is based on rodents' natural aversion to bright light, is another important parameter for evaluating anxiolytic activity. The number of changes between light and dark compartments, as well as the amount of time spent in the light arena, decide anxiety indices (Granziera *et al.,* 2013; Zehravi, Maqbool and Ara, 2021).

First day study:

The time spent in the light box arena increased significantly when the hydroalcholic extract of *Cuscuta reflexa* at 400mg/kg was used in this model of anxiolytic analysis (172±6.01**) compared to the control group (117.518±0.56). Nevertheless, the extract's response was lower than that of the control group (182.33±4.33**)

Based on the anxiety studies it was observed that:

- In the EPM model, the amount of open arm enteries and time spent in open arm were higher when the hydroalcholic extract of *Cuscuta reflexa* was 400mg/kg.

- In a light/dark arena test, it was discovered that a 400mg/kg hydroalcholic extract of *Cuscuta reflexa* increased the amount of time spent in the light arena.

The final outcome of this study confirms the presence of antianxiety properties in the *Cuscuta reflexa*. Flavanoids were discovered in abundance in the fruit. Preliminary phytochemical research also revealed the presence of Flavaonids, tannins, phytosterols, alkaloids, and other compounds that may be responsible for the anxiolytic impact. As a consequence, it is essential to isolate the plant phytoconstituent responsible for the anxiolytic effect, as well as to investigate the mechanism of its antianxiety action. Furthermore, the plant is one-of-a-kind, with just a few phytochemical studies conducted on it. The active moiety responsible for it can be identified and purified using NMR, Mass, and IR for future study.

Critical perspective

Science and technology have contributed significantly to the improvement of humanity's standard of living. On the other hand, modern life stress and its associated hardships and tribulations are to blame for the growth in the occurrence of psychiatric diseases. Anxiety disorders impact 15 to 20% of medical clinic patients, making them the most frequent mental illnesses in the general population. The presence and kind of precipitants, as well as the duration and course of primary anxiety disorders, are all classified. Although anxiety disorders can present with somatic symptoms in the absence of a diagnosable medical condition, a third of medical patients who report anxiety have an organic aetiology for their psychiatric symptoms. Psychopharmacological breakthroughs have flooded the market with specialized drugs. For anxiety, depression, epilepsy, and insomnia, benzodiazepines (diazepam, nitrazepam, lorazepam, and alprazolam, among others) are the most widely prescribed synthetic drugs. Long-term use of benzodiazepines, on the other hand, has a number of negative side effects, including hampering of cognitive function, physical dependency, and tolerance. Benzodiazepines have deleterious effects on the respiratory, digestive, and immunological systems of the body, as well as the potential for addiction, and long-term use of benzodiazepines is generally more harmful. The medicinal plant *Cuscuta reflexa* is very valuable. *C. reflexa* is a parasitic weed plant that kills a large number of crop plants each year. *Cuscuta reflexa* is still known as the "wonder medicinal plant" because various chemical compounds with therapeutic characteristics have been extracted from it. Although *Cuscuta reflexa i*s not well-known in the urban world, it is well-known among rural people in various Indian states. In India and overseas, there are very few research activity in this plant. This plant's many parts are used to treat various ailments. More research on the plant is needed so that more formulations can be offered and employed in the treatment of various ailments. Anti-inflammatory, cytotoxic, antipyretic, hepatoprotective, anticonvulsant, nematicide, anti-androgenic, hypocholestrolemic, antiandrogenic, hemolytic, diuretic, dermatigenic, immunostimulant, antiarthritic, antiasthma, and anticancer activities have all been reported in *Aftimoon* pharmacological studies. The Unani system of medicine is based on a set of holistic principles that assist a practitioner turn a diagnosis into a treatment plan. For treating a patient, the Unani system of medicine employs a variety of techniques. It adheres to a holistic healing philosophy. As a result, it integrates a variety of therapies for curing not only the sickness but also the patient's general health. Herbal medications, which are made from natural things, are used for internal treatment. Although there is growing evidence of the effectiveness of herbs and their preparations in treating anxiety disorders, translating these findings into effective treatment for patients is hampered by a lack of understanding of the chemical composition of the products, a lack of standardization of these preparations, and a scarcity of well-controlled studies. Psychotherapy, nutrition treatment, or medication can be used alone or in combination to treat abnormal or pathological anxiety that has become unbearable and damaging to one's health. Herbal medicines may have a function in the treatment of anxiety disorders, according to preliminary findings, but more research is needed. However, this study is only the first step in that direction, and more research is needed to confirm *Aftimoon Hindi*'s anti-anxiety (*Izterab-e-Nafsani*) effect (*Cuscuta reflexa* Roxb.)

CONCLUSION

The current thesis, titled "Phytochemical Standardization and Anti-Anxiety (*Izterab-e-Nafsani*) Study of *Aftimoon Hindi* (*Cuscuta reflexa* Roxb.) On Animal Model," was conducted at the Regional Research Institute of Unani Medicine (RRIUM) and University of Kashmir-department Srinagar's of *Ilmul Advia*. The procedure had to be accepted ethically before the review could begin. Swiss Albino Mice of both genders were used in the experiment. GAD is a disorder characterised by excessive anxiety and concern over a wide range of events or feelings that the patient perceives to be unimportant or inappropriate. People with GAD are more likely to have other mood and anxiety disorders. While there have been some treatment efficacy trials, there have been few effectiveness trials with broadly representative samples. Non-directive psychotherapy and no medication outperformed cognitive behaviour therapy. As a result, anxiety medication is better than no treatment, and its effectiveness is comparable to cognitive behaviour therapy. Antidepressants, benzodiazepines, buspirone, and kava are all powerful, but they all have side effects. *Cuscuta reflexa* Roxb, a member of the *Cuscutaceae* family, is known as *aftimoon* in Unani. For decades, Unani doctors have used it to treat melancholia, schizophrenia, and epilepsy, among other ailments. It's also used to treat a variety of ailments, including hepatitis, palpitations, and skin problems. In comparison to the regular prescription, the hydro alcoholic extract of a higher dose of the test drug showed excellent anxiolytic efficacy and no side effects. These findings indicate that a double dose of *Aftimoon* (400 mg/kg body weight) has an anti-anxiety effect comparable to a normal medication.

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