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Evaluation of anxiolytic activity of Rasan (*Inula racemosa* Hook. f.) roots in wistar rats

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Abstract
Inula racemosa Hook. f., commonly known as *Rasan* in the Unani system of medicine, belongs to the family Asteraceae and has been used by Unani physicians since ages for the treatment of various ailments such as arthritis (*Waja'ul-Mafasil*), ascites (*Istisqa*), bronchitis, palpitation (*Khafaqan*), Otitis (*Waja' ul-Udhun*), skin disorder (*Jildi amraz*),and neurological disorders (*Amraz-e-nafsaniya*) like Mania (*waswas*), Insomnia (*sahr*), melancholia (*Malankhuliya*), sciatica (*Niqris*), migraine (*Shaqiqa*) and anxiety (*Izterab-e-Nafsani*). Despite a long history of uses, no scientific pharmacological evaluation has ever been carried out on this plant. Thus, the present study was envisaged to evaluate the anti- anxiety effects of the extract from *Rasan* (*Inula racemosa* Hook. f.) using various models of anxiety. Hydroalcoholic extract of *Rasan* (*Inula racemosa* Hook. f.) were evaluated for anxiolytic effects using elevated plus maze test (EPM) and light-dark box model of anxiety at a dose 200mg/kg and 400mg/kg respectively. Among the various fractions tested, maximum anxiolytic activity was observed by hydro-alcoholic extract (400mg/kg) fraction which was at par with that of diazepam. The results of present investigation provide significant anxiolytic activity observed with hydro-alcoholic extract of the higher dose of the test drug when compared to standard drug and hence justifying the use of *Rasan* (*Inula racemosa* hook.f.) in Unani system of medicine for various neuropsychological disorders (*Amraz-e-nafsaniy*).

Keywords: *Rasan* (*Inula racemosa* Hook. f.), anxiety disorder, mania, unani medicine

1. Introduction
Anxiety, fear and worry are all completely natural human feelings. Anxiety is a complex progressive behavioural and physiological alteration of the organism, which ultimately leads to wide variety of central nervous system (CNS) disorders [1]. It is an emotional state, unpleasant in nature, associated with uneasiness, discomfort and concern or fear about some defined or undefined future threat. Some degree of anxiety is a part of normal life. Anxiety is implicated in a number of psychiatric disorders, such as depression, panic attacks, phobias, generalized anxiety disorder, obsessive-compulsive disorder and post-traumatic stress disorder [2]. If these feelings occur and endure for an extended period, it affects both physical and mental health. In addition to individual genetic factors external influences, such as nutrition, smoking, alcohol, socioeconomic status, environmental conditions etc. can strongly contribute to its anticipated appearance [3]. This leads to clinical anxiety disorders. Anxiety is a universal phenomenon and to experience it in appropriate circumstances is the normal response. It may serve to enhance the vigilance and drive. However, if anxiety symptoms are frequent and persist in severe form, they are a cause of distress/suffering and markedly impair performance. It should be treated with drugs only when excessive and disabling in its own right. The life time prevalence of panic attacks (a form of anxiety disorder) is around 7-9% in most countries and 1% alone in India with the prevalence of generalized anxiety disorder is very high i.e. 8.5% in the general population [4]. Anxiety disorders affect 16.6% of population worldwide [5] and numerous efforts have been made to understand the pathophysiology of the disease and treatments.There are many types of treatment available to treat anxiety disorders. This article outlines more common herbal remedies to treat anxiety disorders.
Anxiety disorders, as such, are not mentioned in Unani literature but their symptoms either separate or with a cluster of others in different diseases are described under various headings like Melancholia (*malikholia*), Mania (*waswas*), Insomnia(*sahar*), *"tawahhush", "hizyan", "ishque" and "khafqan"* (Palpitation) [6]. Ibn Sina described several psychiatric disorders including the so-called disorder of affection, which he considered as a fanatical disorder resembling severe depression [7]. The term *"iztirab"* is used for anxiety in Arabic and Unani texts, and the word *"Nafsani"* is added to *iztirab* to specify its status [8]. *Iztirab-e-nafsani*

(Anxiety) and depression is a disabling illness the risk of suicide. Compared with other professions, healthcare workers were associated with higher risk for poor sleep quality younger people spending too much time thinking about the outbreak, and healthcare workers were at high risk of mental illness [10]. Pharmacological treatment for anxiety disorder consists of the use of benzodiazepines and antidepressant drugs; however, although these treatments showing clinical efficacy, they have several problems. Benzodiazepines can lead to disturbing effects, such as amnesia, dependence liability, and sedation. Similarly, the other antidepressants can lead to sexual dysfunction, insomnia, and gastrointestinal disturbances [11, 12]. Thus, there is a need for the development of new anxiolytic drugs that lack these effects. In this context, medicinal plants have a potent and safe role as anxiolytic [13].

Rasan (*Inula racemosa* Hook.f.) 'a perennial herb and is found only in the hilly regions of temperate to alpine western Himalayas (1600-4200 m) [14]; It is a less-known drug of Unani system of medicine [6], which belongs to the Asteraceae family [15]. This drug is mentioned in Unani literature as *Rasan* or *Zanjabeel Shami* [6] and in Ayurvedic literature, referred to as Pushkarmuul [16]. Traditional *Rasan* have been used for the treatment of various ailments like melancholia, migraine**,** convulsions, phlegmatic diseases, sciatica, gout, as diuretic, and management of amenorrhea, leucorrhea, anal fissure [6, 17]. Pharmacologically *Rasan* (*Inula racemosa* Hook. f.) exhibits cardioprotective, analgesic, anti-inflammatory, anti-allergic, anti-bacteria, anti-fungal, adaptogenic, anti asthmatic, hepatoprotective, hypoglycaemic, cytotoxic, anti-platelet, radioprotective, complexion promoter, mosquito larvicidal, anthelmintic and antioxidant [18]. Despite a long history of use *Rasan* (*Inula racemosa* Hook.f.) as traditional medicine for the treatment of various ailments, especially in CNS disorders the plant has never been subjected to anxiety activity. Thus, it was considered worthwhile to evaluate *Rasan* (*Inula racemosa* Hook. f.) for anti-anxiety activity.

2. Materials and Methods

2.1 Plant Material

The roots of *Inula racemosa* were collected from the Nyle Village Gurez, Srinagar, Jammu and Kashmir India. The plant was identified and authenticated by Botanist at RRIUM, Srinagar, Department of Taxonomy, wide Specimen Voucher no.5859.

2.2 Organoleptic characters and morphological study of *Inula racemosa* Hook. f

The identity and authenticity of drug material was revealed by studying the organlptic characters like colour, smell, taste and texture of the root as well as morphology and microscopy [18].

2.3 Physicochemical studies

Physicochemical characteristics like moisture content, ash values(total ash, acid insoluble ash, water soluble ash, sulphated ash) and extractive values both exhaustive and sequential extraction were studied according to the reported method [18].

2.4 Phytochemical analysis

Preliminary phytochemical screening of ethanol and methanol extract of test drug roots were performed for the presence or absence of different chemical constituents like carbohydrates, alkaloids, flavonoids, phenolics, tannins, steroids, saponins, anthraquinone, glycosides, fixed oils and fats [19].

2.5 Preparation of extract

The roots of *Inula racemosa* were thoroughly washed and shade dried for 7 days. The dried roots were pulverized using mechanical grinder. The coarsely powder (about 220 g) was first defatted in petroleum ether in a closed glass jar for 72 h and then it was extracted with hydro-alcoholic solution, having respective percentage ratio of (30:70), by cold maceration for 24 h. The macerated mixture was filtered through muslin cloth and evaporated to dryness at 40 °C using rotary vacuum evaporator at (Yamato Rotary Evaporator 300, Japan). After maceration, the solvent was distilled off and the extract was concentrated for 2 h of incubation at 37 °C in a water bath and the yield percentage was 25.18% *w/w* and was stored at -4 °C till further use.

3. Pharmacological evaluation

3.1 Animals

The study was carried out on Wistar rat of either sex, weighing 75-100 gm. Institutional Ethical Committee had approved the protocol (IAEC-Approval no: KU/2017/16). Animals were housed individually in suspended stainless-steel cages, provided with pelleted feed procured from Pranov Agro Industries, New Delhi and filtered/sterilized water (Aqua Guard KENT RO) *ad libitum* and maintained in an air-conditioned room at 22±3 °C humidity of 50±20%, 12-h light (8:00–20:00)/& dark (20:00–8:00) cycle and ventilation at 10-15 times/h.

3.2 Dose of the test drug and grouping of animals

The dose of the test drug for rat was calculated by extrapolating human therapeutic dose [20] with suitable conversion factor of 7 (to accommodate the body surface area of rats) [21]. The animals were divided into four groups, with six animals in each group. Group I (Control group) were administered with 0.5% CMC orally; Group II received hydro-alcoholic extract 200mg/Kg; Group III received hydro-alcoholic extract 400mg/Kg and Group IV (standard control) received standard drug Diazepam 1 mg/kg IP [19]. Diazepam 1 mg/kg IP was used as standard anxiolytic agent 0.5% Carboxymethyl cellulose (CMC) was used as vehicle for making different test doses of hydro-alcoholic extract of *Rasan* (*Inula racemosa*).

3.3 Models for evaluating the anti-anxiety activity

Two models were used for evaluating the anti-anxiety activity:

1) Elevated plus-maze [21].
2) Light and dark arena [22].

3.3.1 Elevated plus-maze (EPM)

EPM is currently one of the most widely used models for evaluating anti-anxiety [21, 23]. The EPM apparatus consisted of two open arms (35cm x 5cm) and two closed arms (30cm x 5cm x 15cm) that extended from a common central platform (5cm x 5cm) to form a plus sign. A slightly raised edge on the open arms (0.25cm) provided an additional grip for the animals. The maze floor and the closed arms were covered with black adhesive tape and elevated to a height of 50 cm above floor level by a single central support. Animals were given vehicle; diazepam and oral doses of the plant extract 60 min before their placement on the EPM. The number of entries and the time spent in the open and closed arms were recorded during a 5minute test period. A similar procedure was carried out on the seventh day for chronic study. The

percentage of open arm entries (100 x open/total entries) was calculated for each animal.

The percentage of time spent in the open arm was determined as follows:

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

3.3.2 Light and dark test

The apparatus consisted of a rectangular box (45x27x27), partitioned into two compartments connected by a 7.5x7.5 cm opening in the wall between compartments. One compartment was painted black and covered with a roof. The other compartment had no roof and was brightly illuminated by a 60W bulb located above the box. Rats were given vehicle, diazepam, and oral doses of extracts and after 60 minutes, each animal was placed in the centre of the light compartment and was observed for 5 min [22, 23].

The time spent in the open (white/light) compartment was recorded. A similar procedure was carried out on the seventh day for chronic study. The percentage of time spent in the light compartment was determined as follows:

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

4. Statistical Analysis

The data were analyzed using Graphpad software. ANOVA one-way with Post hoc Kruskal Wallis test with Dunn's comparison was used. The values were expressed as mean ± SEM. Difference between the means was considered significant at $P<0.05$.

5. Results

5.1 Organoleptic characters and morphological study of *Inula racemosa* Hook.f

Organoleptic evaluation of root found that it is having externally greyish brown and internally yellowish brown colour, camphoraceous and aromatic smell and surface was rough with longitudinal striations and cracks.

Thin walled polygonal tabular lignified cork cells, beneath rounded-rectangular parenchymatous cells with medullary cells being somewhat elongated radially, the rays of phloem consist of groups of yellowish and slightly lignified thick walled fibres alternating with sieve-tissue, the group of xylem and xylem vessel similar to those of the phloem but more strongly lignified were seen in TS of root of *Inula racemosa* Hook. Vascular region shows presence of elongated oil cells.

5.2 Physicochemical studies

Physicochemical analysis results of root of *Inula racemosa* Hook were mentioned in table 1.

Table 1: Physicochemical analysis results of root of *Inula racemosa* Hook were mentioned

S. no.	Parameters	Mean±SEM
1	Moisture content	13.8%
4	Total ash %	4.3%
5	Acid soluble ash %	0.42%
6	Water soluble ash %	0.14%
7	Water soluble extract	28.0%
8	Alcohol soluble extract	16.0%

Table 2: Phytochemical screening of bioactive crude extract and its active fraction of roots of Inula racemosa Hook.

Sr. No	Test	Bioactive extract* (ethanolic extract)	Bioactive fraction* (methanolic fraction)
1	**Test for Carbohydrates**		
	Molish test	+	_
	Fehling's Test	+	_
2	Test for proteins		
	Millions test	_	_
3	**Test for fats and oils**		
	Solubility test	_	_
	Filter paper test	_	_
4	**Test for Terpenoids**		
		+	+
5	**Test for Anthraquinones**		
		+	+
6	**Test for Phytosterols**		
	Salkowaski test	+	+
	Lieberman-Burchard test	+	+
	Sulphur test	+	+
7	**Test for Alkaloids**		
	Mayer's reagent test	_	_
	Dragendorff's reagent test	_	_
	Hager's test	_	_
	Wagners test	_	_
8	**Test for glycoside**		
	Legal test	_	_
9	**Test for Flavonoids**		
	Shinoda Test	+	+
10	**Test for Phenols**		
		+	+
11.	**Test for Tannins**		
		+	+
12	**Test for Saponins**		
		+	+

Remarks: + (Present) ; – (Absent). *Bioactive extract/ fraction

5.3 Elevated Plus-Maze model (EPM)

EPM is currently one of the most widely used models of animal anxiety. EPM is based on the natural aversion of rodents for open spaces and uses the conflict between aversion and exploration to open spaces. Provoked behaviour profiles in the EPM appear to include elements of neophobia, exploration, and approach/avoidance conflict [24, 25]. An increase in open arm parameters is the most important representative indices of anxiolytic activity. Time spent on the central platform appears to be related to decision making and or risk assessment and the total arm entries are measure reflecting changes in anxiety or general activity [26].

In the EPM, the results showed that hydro-alcoholic extract of root at a dose of 200 mg/kg b.w. showed the most significant time spent in the open arm i.e., 71±16.217 sec (p>0.05) on the 1st day and 75.66±12.336 sec (p<0.05) on the 7th day as compared with control dose of 0.5%CMC, which showed 4.5±5.025 sec on the 1st day and 25.33±2.616 sec on the 7th day in the open arm respectively. The hydro-alcoholic extract of root at a dose of 400 mg/kg b.w. showed the most significant time spent in the open arm 85.66±7 sec (p<0.05) on the 1st day and 88.16±11.47 sec (p<0.01) on the 7th day as compared with control at a dose of 0.5% CMC, which showed 4.5±5.025 sec on the 1st day and 25.33±2.616sec on the 7th day in the open arm respectively. The results indicate that hydro-alcoholic extract of root at a dose of 200 mg/kg b.w and hydro-alcoholic extract of root at a dose of 400 mg/kg b.w. showed statistically significant anti-anxiety activity as compared to the standard drug Diazepam, which showed 94.83±7.634 sec (p<0.05) on the 1st day and 104±12.482 sec (p<0.001) on the 7th day of the time spent on the EPM apparatus in the open arm respectively.

Table 3: Anxiety related behaviour using Elevated Plus-Maze model (EPM):

Groups	Days	Time Spent (Sec)		Number of Entries	
		Open arm	Closed arm	Open arm	Closed arm
Control	1st	4.5±5.025	251.83±8.882	3.33±1.211	6.33±0.421
	7th	25.33±2.616	233±9.118	3.833±0.307	7±0.365
Diazepam (1mg/Kg)	1st	94.83±7.634*	180.838±7.53**	4.66±0.33**	4.166±0.307**
	7th	104±12.482***	170.5±7.388***	6.33±0.333***	3.166±0.477***
Extract (200mg/Kg)	1st	71±16.217ns	229.5±16.01ns	4±0.25ns	5.166±0.307ns
	7th	75.66±12.336*	196±12.482ns	5±0.258*	4.5±0.806*
Extract (400mg/Kg)	1st	85.66±7*	199.66±16.99*	5.166±0.166**	4.66±0.333*
	7th	88.16±11.47**	83.83±8.3**	5.333±0.21**	4±0.516**

Note: 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.

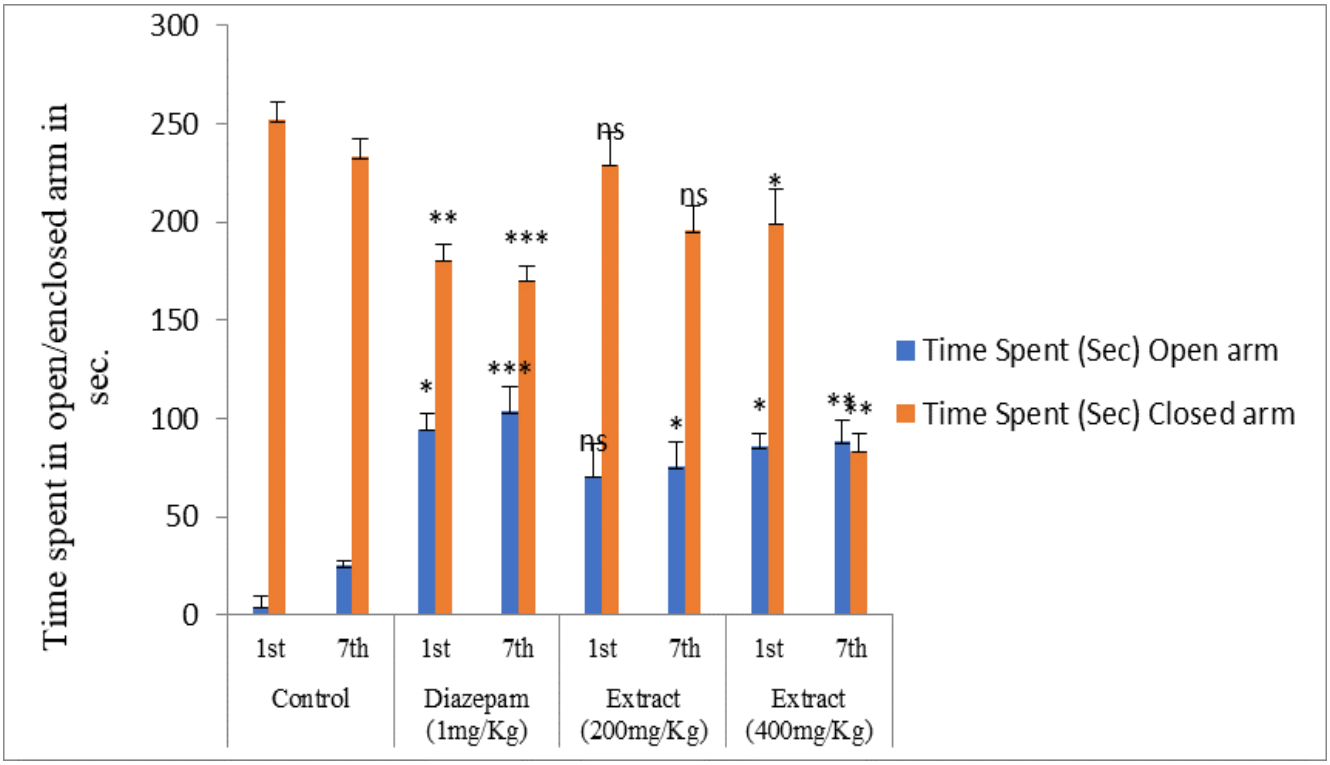

Fig 1: Graphical representation of the effect of Hydro-alcoholic root extract of *Inula racemosa* on Time spent in open/closed arm in the EPM model

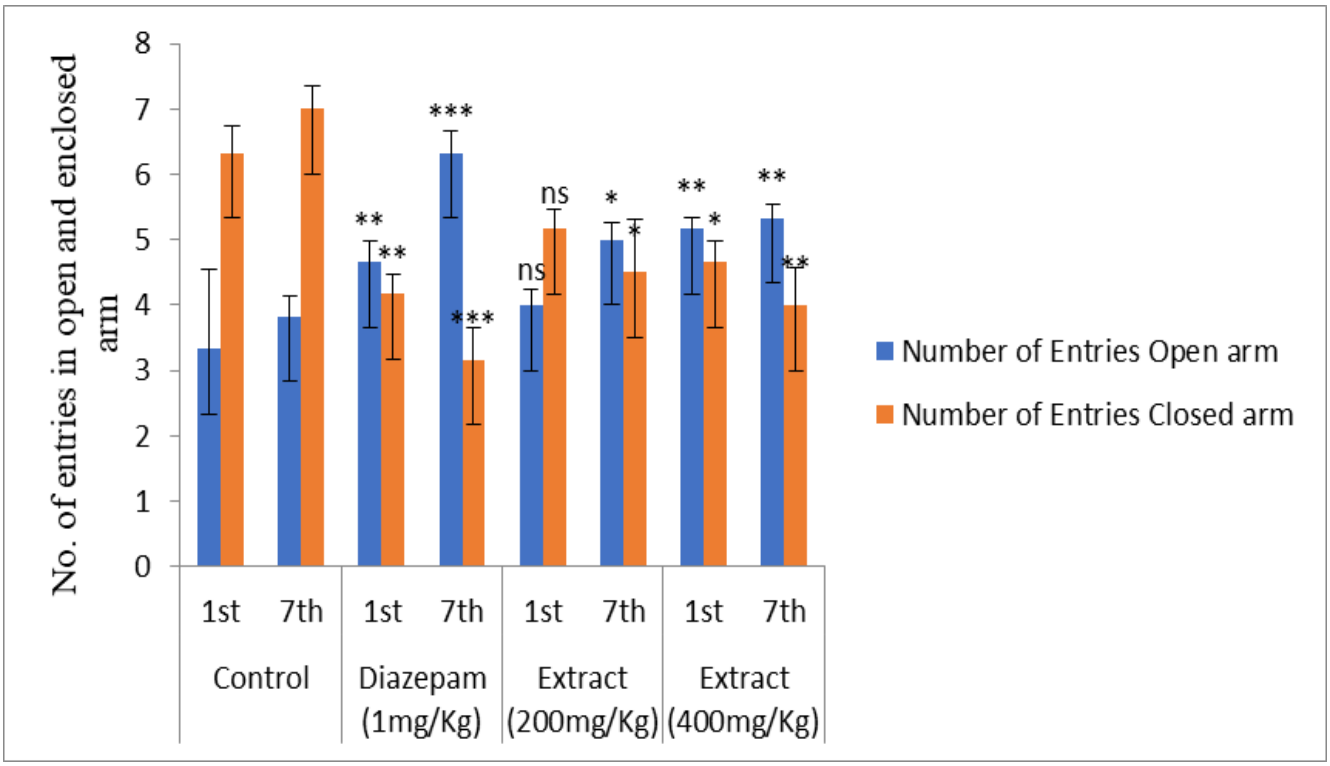

Fig 2: Graphical representation of the effect of Hydro-alcoholic root extract of *Inula racemosa* on open/closed arm entries in the EPM model

5.4 Light and dark test

The light/dark has been used as an anxiolytic activity test in rodents. Anxiolytics have been found to increase the time spent in the light zone whereas anxiogenic drugs decrease it [27]. Rats also prefer dark environments [28, 29] which makes this parameter potentially useful to assess the effects of anxiolytic activity.

In the light and dark test, the results showed that hydro-alcoholic extract of root at a dose of 200 mg/kg b.w. showed the most significant time spent in lighted area 57.5±12.01ns sec on the 1st day and 65±14.895nssec on the 7th day as compared with control at a dose of 0.5% CMC which showed 58.5±5.892 sec on the 1st day and 31.16±2.173sec on 7th day in the lighted area. The hydro-alcoholic extract of root at a dose of 400 mg/kg b.w. showed the highest time spent in the lighted area i.e. 95.166±8.67 sec (p<0.05) on the 1st day and 88.66±4.66 sec (p<0.01) on the 7th day as compared control at a dose of 0.5% CMC, which showed 58.5±5.892 sec on the 1st day and 31.16±2.173sec on the 7th day in the lighted area. The result indicates that hydro-alcoholic extract of root at a dose of 400 mg/kg b.w. showed statistically significant anti-anxiety activity as compared to the standard drug Diazepam, which showed 106.33±6.396 sec (p<0.01) on the 1st day and 109.5±10.68 sec (p<0.001) on the 7th day in the lighted area respectively.

Table 4: Light and Dark Test

Groups	Days	Time Spent (Sec)		Number of Entries	
		Light arena	Dark arena	Light arena	Dark arena
Control	1st	58.5±5.892	254.833±10.489	3.166±0.703	6.33±0.421
	7th	31.16±2.173	259.833±10.57	3.166±0.6	6.833±0.307
Diazepam (1 mg/Kg)	1st	106.33±6.396**	192.33±12.019**	6.166±0.477**	4.33±0.816**
	7th	109.5±10.68***	173.33±13.793***	6.833±0.307***	3.66±0.421***
Extract (200mg/Kg)	1st	57.5±12.01ns	231.166±12.352ns	4.33±0.494ns	5.66±0.218ns
	7th	65±14.895ns	231.833±13.793ns	4.83±0.654ns	4.16±0.477**
Extract (400mg/Kg)	1st	95.166±8.67*	205.5±6.07*	5.5±0.22*	4.66±0.33**
	7th	88.66±4.66**	189.66±9.49**	5.83±0.54*	4±0.516***

Note: 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 Hydro-alcoholic extract of *Inularacemosa*

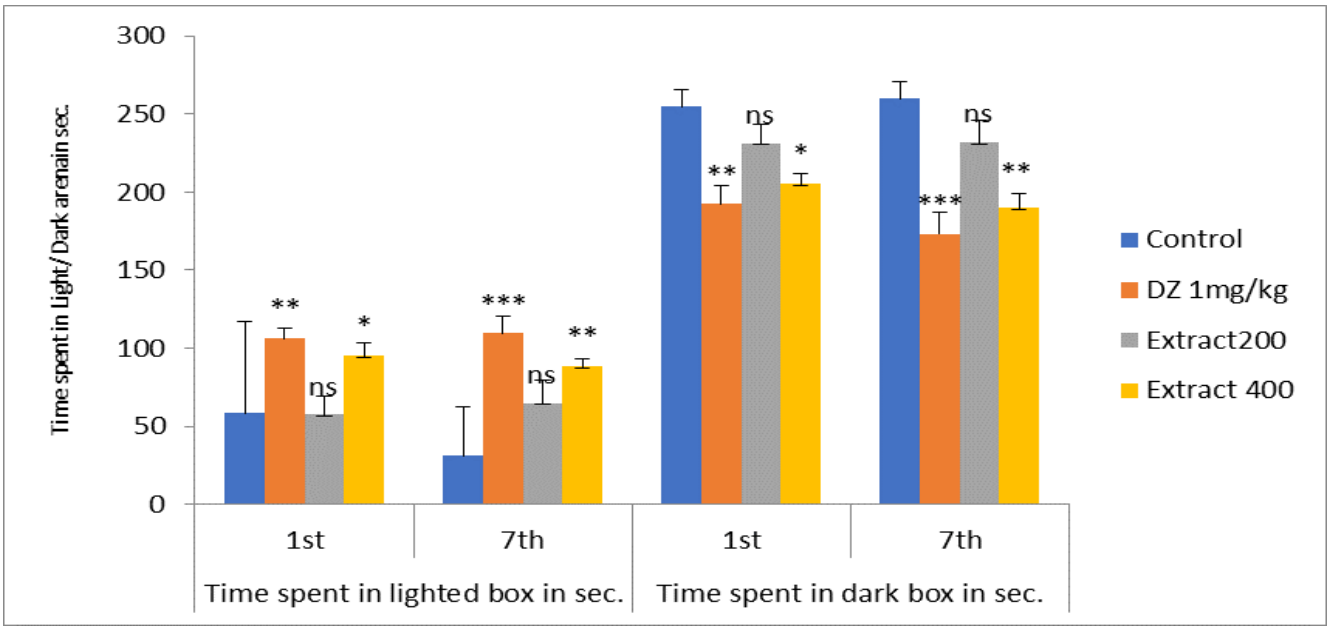

Fig 3: Graphical representation of the effect of extract of *Inula Racemosa on* time spent in Light and dark arena model

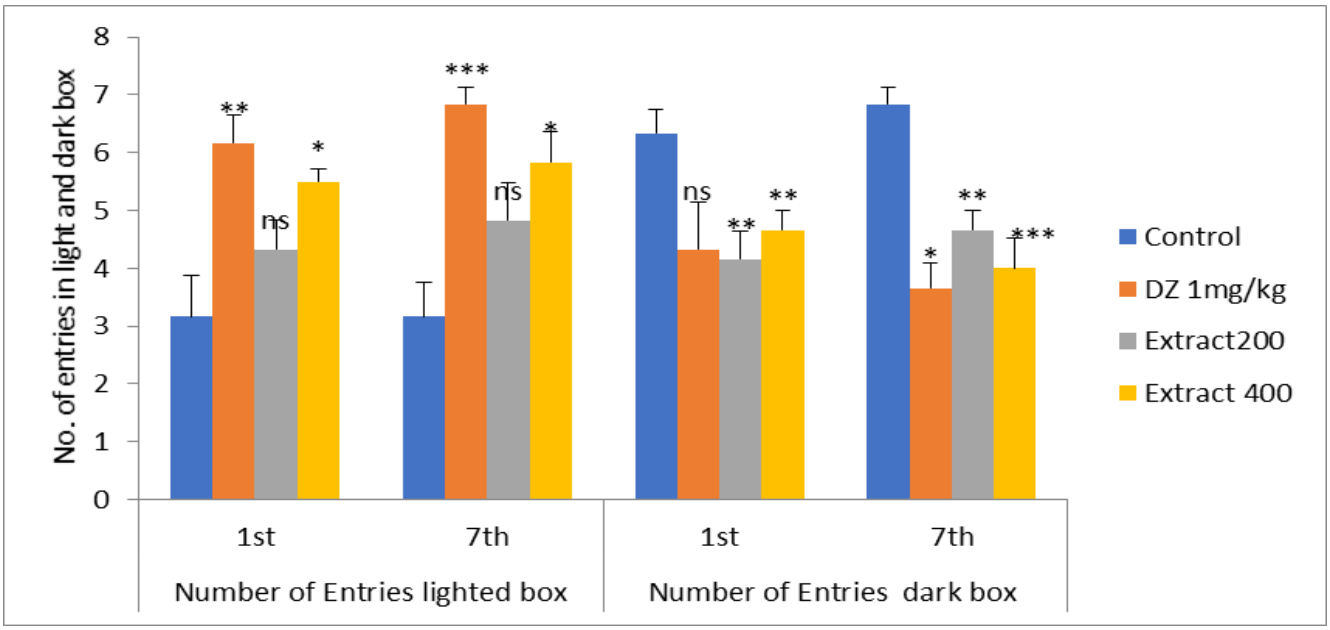

Fig 4: Graphical representation of effect of extract of *Inula racemosa*on time spent in Light and dark arena model on the number of entries in light and dark box.

6. Discussion

In the management of anxiety disorder and its acute symptoms, anamalgamation of therapeutic intrusions is mostly specified. Anxiolytics are a part of treatment of anxiety, beside a psychotherapeutic approach. Benzodiazepines are the most commonly given for the last 40 years to treat several forms of anxiety; however, they have prominent side effects such as sedation, myorelaxation, ataxia and amnesia, and can cause pharmacological dependence [29]. Unani medicines have quite long been used as the treatment of anxiety disorders and relief by the traditional healers with minimum reported side effects. In the current work we examined the anxiolytic effects of hydro-alcoholic extract of *Inula racemosa* Hook, using the light/dark test and the elevated plus maze test. Furthermore, the effects of *Inula racemosa* Hook and diazepam on these animal models were compared to determine whether the behavioural profile *Inula racemosa* Hook differed from an established anxiolytic drug.

In the light/dark test, anxiety is generated by the conflict between the tendency to explore and the initial tendency to avoid the unfamiliar [24] and can be evaluated according to the number of transitions in to and the time spent in the light chamber [25, 26] where in increase in these parameters is considered to reflect anxiolytic-like properties. Our results showed that the extract (400 mg/kg) increased time spent in the light chamber, suggesting anxiolytic action.

The number of open arm entries and time spent in open arm in the case of the EPM model was higher incase of hydro-alcoholic extract of *Inularacemosa* at 400mg/kg. The results observed in this study confirm the presence of the anti-anxiety potential of the *Inularacemosa*. The plant has been reported to be rich in Alantolactone, Isoalantolactone, Alantol and Inulinadrenaline, and serotonin which may be responsible for the anxiolytic effect [30]. Hence there is a need for the isolation of the plant phytoconstituent responsible for the anxiolytic effect and also to study the mechanism of its anti-anxiety behavior.

7. Conclusion

It is concluded from the current investigations that *Rasan* (*Inula racemosa* Hook. f.), exhibit significant antianxiety and antidepressant activity, thereby validating the traditional use of the plant in the treatment of mental disorders like anxiety. Future prospects of the current investigation include isolation and characterization of bioactive constituent(s) from Hydroalcoholic extract of *Rasan* (*Inula racemosa* hook. f.) and mechanism of action involved in anxiolytic activity.

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