International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Bio Evaluation of Mirabilis Jalapa Leaves Extract for Anxiolytic Activity in Animal Model

Ms. Varsha M. Kharat[1], Dr. Ranajit D. Tijare[2], Ms. Ambika D. Nagarbhadiya[3], D. Nagarbhadiya[4]

[1,3]Assistant Professor, Dept. of Pharmacology, D.B.K.S.S. Ishwar Deshmukh Institute Of Pharmacy, Digras
[2]Principal, Dept. of Pharmacognosy, D.B.K.S.S. Ishwar Deshmukh Institute Of Pharmacy, Digras

Abstract
Anxiety is an exaggerated feeling of apprehension, uncertainty and fear. It is an unpleasant state of tension with an anticipation of imminent danger. It may be regarded as a particular form of behavioral inhibition that occurs in response to environmental events that are Novel. In recent times, there are many indigenous plants have beneficial properties to treat mental disease and psychic complaints. *Mirabilis Jalapa Linn* (family- Nyctanginaceae) is one of the plants that used for health care and medicinal purpose for thousands of year's .It has traditionally been used in the treatment of gastrointestinal disorders, muscle pain, abdominal colic, and diarrhea. However, studies have evidenced its antibacterial, antiviral, antioxidant, anti-diabetic, anti-inflammatory, anti-stress and many other activities. The purpose of this study was to prepare & evaluate Methanolic extract of leaves of mirabilis Jalapa for the treatment of anxiety in animal models. The dried leaves was extracted with methanol separately and administered the dose of 200mg/kg and employed in elevated plus maze test and open field test with 2mg/kg i.p.of Diazepam as a standard drug to assess the anxiolytic activity. The result reveled that methanol leaf extract was more impetus due to high amount of flavonoid, phenolic compound, steroids; terpenoid contents possess tremendous anti-anxiety activity. This study suggested that the *M.Jalapa* is much more active compound consistent medicinal plant to derive a potent drug against Anxiety

Keywords: Anxiolytic, Mirabilis Jalapa, Elevated Plus Maze, Methanolic

Introduction:

A mental health disorder characterized by feeling of anxiety, worry and fear that are strong enough to interfere with one's daily activities. Occasional anxiety is a normal part of life. Many people worry about things such as money, health and family problems. But anxiety disorder involves more than temporary worry or fear. Anxiety disorder involves repeated episodes of sudden feeling of intense anxiety and fear or terror that reach to peak within minutes. Over 40 billion people in U.S. have an anxiety disorder. Anxiety disorder affect one-eighth of population worldwide. There are several types of anxiety disorders including generalized anxiety disorder, panic disorder, social anxiety disorder and various phobia related disorders.

In Recent times, very few anti-anxiety drugs are available in market; furthermore many anxiety patients faced multifarious difficulties associated with anti-anxiety drugs. And hence demand of herbal medicine

is increasing due to their wide efficacy, and least side effect. Different medicinal plants have been used for thousands of years to treat, prevent and control of various diseases throughout the world. Our people use lots of medicine for their treatment and use.

Mirabilis Jalapa Linn (family- Nyctaginaceae) is one of the plants that used for health care and medicinal purpose for thousands of years. It is a perennial bushy herb promulgate by flowers or leaves, a native of India and commonly known as 'four-o-clock'. It has traditionally been used in the treatment of gastrointestinal disorders, muscle pain, abdominal colic, and diarrhea. However, studies have evidenced its antibacterial, antiviral, antioxidant, anti-diabetic, anti-inflammatory, anti-stress and many other activities. Phytochemical analysis of leaf parts of *M. Jalapa* revealed the presence of potent secondary metabolites in sufficient extent.

Materials and methods

Diazepam as a standard drug, Methanolic extracts of leaves of *mirabilis Jalapa* Linn

Collection and authentication of plant material of *Mirabilis Jalapa* leaves:-

The leaves of *Mirabilis Jalapa* belonging to family-Nyctaginaceae were collected in the month of September from the local area of Yavatmal district, Maharashtra, India.

Extraction of *Mirabilis Jalapa* leaves

The fresh leaves of *Mirabilis Jalapa* were collected and washed under running tap water and dried in shade.

The preparation of extract is done by using the process of maceration. Use methanol as solvent.

The plant drug is used to dry under the shade and ready for coarse grinding by using the grinder. Once the powder is set in the maceration assembly it is allow for the 24 hrs under the pressured temperature for the extraction of crude drug. The methanol allow to the extraction on crude drug. After 24 hrs. The extract is allowed to concentrate & dry for the utilization.

Finally, the extract was transferred to air tight container and stored at cool place for further pharmacological activity.

Phytochemical Screening

In the investigation of phytochemical analysis of *Mirabilis Jalapa* leaves shows the presence of various Phytoconstituents.

Sr No.	Phytoconstituent	Test performed and reagents	Extract results
		i)Mayer'stest	+
1	Alkaloids	ii)Dragondroffstest iii)Wagner'stest	+ +
		iv)Hager'stest	+
		i)Benedict'stest	+
2	Carbohydrates	ii)Fehling'stest	+
		iii)Molicsh's test	+
3	Glycosides	i)Keller-killani test	_

4	Tannins	i)Gelatin test ii)Lead acetate test	+ +
5	Saponins	i)Foam test ii)Froth test	+ +
		i)Ferric chloride test	+
6	Flavonoids	ii)Alkaline reagent test	+
		iii)Leadacetatetest	+
7	Steroids	i)Liebermann-Burchard'test	+
8	Terpenoids	i)Salkowski's test	+
9	Phlobatannins	i) Hydrochloric acid (1%)	+
10	Anthraquinones	i)Borntranger'stest	_

Table1: Phytochemical Screeningofmethanolicextractof*MirabilisJalapa*Plant

Screening of Anxiolytic Activity

1. Elevated Plus-Maze Test

The apparatus was made of Plexi glass and consisted of two open arms (15 cm x 5 cm x 1 cm) and two enclosed arms (15 cm x 15 cm x 5 cm). The arms extended from a central platform (5 x 5 cm2) forming a plus-sign with like arms opposite each other which was elevated 60cm from the floor. Male Wister rats weighing 180- 200g were grouped into six (n=6). Four groups (III, IV,V, VI) were treated with the extracts (200and 400 mg/kg p.o), other groups (II) treated with Diazepam 5mg/kg and the last grouped administered with 0.5% of CMC to serve as control (I) and 1 hour after oral treatment with the extract and vehicle, rat were placed individually in succession in the central platform of the maze for 5 minutes and their behavioral parameters like number of entries and time spent in open arms were recorded. An arm entry was defined when all four paws of the rat were in the arm.

2. Open Field Test

The study was conducted according to the method described by Brown et al. with some modifications. The apparatus was made up of plywood measuring 72cm × 72cm × 36cm. On the top of OFT apparatus fix camera to ensure that the mouse under investigation is visible to the observer. The floor, made of cardboard, was divided into 16 equal squares (18cm × 18cm) with blue marker and a central square drawn with brown marker. Here, the cardboard was covered with a transparent Plexiglas. The animals were divided into six groups and Diazepam is a standard drug as mentioned in the previous experiment. Thirty minutes later, each mouse was placed individually at the corner of the arena and its behavior monitored for 5min. The number of squares crossed and number of rearing each rat was recorded. The apparatus was wiped between observations with 70% ethyl alcohol and allowed to dry to remove any olfactory cue.

Result

Phytochemical Evaluation

The phytochemical evaluation results revealed the presence of biologically active secondary metabolites such as flavonoids, Steroids, Phenols, glycoside, Alkaloid. Quercetin is one of the Flavonoids shows ant-

ianxiety effects.

Evaluation of anti-anxiety activity

Elevated Plus Maze Test -

In the Elevated plus maze apparatus (0.5% CMC used as a vehicle) treated male rats had a tendency to prefer to stay in closed arm for a longer period of time than in open arm.Treatment with diazepam (5mg/kg) increased the number of open arm entries ,and time spent in open arm (14.91 ±1.10 sec and 259.10 ±1.40) it suggest that diazepam produces anxiolytic activity .Mirabilis Jalapa produced a dose dependent increase in time spent in open arm along with an increase in number of open arm entries .comparably *Mirabilis Jalapa* leaves extract(200mg/kg) anxiolytic effect was significant to that of diazepam 5mg/kg as 13.31 ±1.12 number of entries and 241.57 ± 1.27 sec time spent in open arm.

Group	Treatment	No.of entries in open arm	Time spent in open arm
I	Control	3.22 ±1.71	13.50 ±1.59
II	Diazepam (5mg/kg)	13.91 ±1.10***	249 ±1.40***
III	MJLE (200mg/kg)	11.31 ±1.32***	213.38 ±1.60***
IV	MJLE(200mg/kg)	13.31 ±1.12***	241.57 ±1.27***

Open field test

The number of square crossed and number of rearing each rat was recorded. The number of times the frequency crosses the grid lines on the grid floor insert with all four of its paws and frequency of rearing behavior where the subject stands on its hind paws were observed .In general unsupported rearing is a better measure of anxiety. In the open field test ,diazepam and extract (*mirabilis Jalapa* leaves 200 mg/kg) treated rat showed significant increase in the number of rearing and number of square crossed during 5 min interval as compared to vehicle treated control group. Whereas anxiolytic effect of *mirabilis Jalapa* leaves extract (200mg/kg) was comparable to that of the diazepam.

Conclusion

The preliminary phytochemical studies were carried out in the methanolic extract of *mirabilis Jalapa* leaves, observed the significant presence of alkaloids, carbohydrates, Flavonoids, phenols and steroids, glycosides and tannins. However on the basis of our experimental result the higher reponseof methanolic leaves extraction of mirabilis Jalapa showed significant anxiolytic activity .The present study is the first evidence of antianxiety properties of *mirabilis jalapa*.it is concluded that neuroprotective effects of mirabilis Jalapa might be due to the presence of tannins, Phenolic compound and Flavonoids.

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