ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 5, Issue 5 , May 2018

Phyto - chemical and anxiolytic effect of methnolic extract of vitis vinifera leaf

Jyoti Singh* , Bishnu Kumar

Assistant Professor, Dept. of Pharmacologey, Ashoka Institute of Technology and Management, Varanasi, UP, India.
AssociateProfessor, GS Ayurvedic Medical College, Ghaziabad , UP, India.

ABSTRACT: *Vitis vinifera* belonging to the family vitaceae is a beneficial medicinal herb, different parts of this plant traditionally used, in particular from fruits, contains an abundant amount of vitamins and minerals. The present study was designed to the anxiolytic activity of methnolic extract of *vitis vinifera* (*MEVV*) by different behavioural models. The leaf of the plant were collected and authenticated. Preliminary phytochemical investigation revealed the presence of various phytoconstituents like phenols and flavonoids. The extract at 100, 200 and 400mg/kg was evaluated with the adult mice by Elevated plus-maze test [EPM] and Open-field test [OFT]. The results of behavioral tests indicated the dose dependent anti-anxiety activity of methnolic extract of *vitis vinifera* (*MEVV*) which is comparable to anxiolytic drug diazepam (2.5 mg/kg). It was concluded that extract of *vitis vinifera* (*MEVV*) showed antianxiety activity. Further studies are needed to identify the anxiolytic mechanism(s) and the phytochemicals responsible for the observed anxiolytic effect of the *vitis vinifera*.

KEYWORDS: *vitis vinifera*, anxiolytic activity, Elevated plus-maze test, Open-field test.

I.INTRODUCTION

Anxiety is a complex progressive behavioral and physiological alteration of the organism, which ultimately leads to wide variety of central nervous system (CNS) disorders, if remain untreated. 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. Some degree of anxiety is a part of normal life. Treatment is needed when it is disproportionate to the situation and excessive. Some psychotics and depressed patients also exhibit pathological anxiety. 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. [1] Approximately two-thirds of the anxious patients respond to the currently available treatments but the magnitude of improvement is still disappointing, besides, they also produce various systemic side effects and exhibit dependence and tolerance on chronic treatment which now have become a major concern about the use of currently used medicines [2]. Herbal medicines are popular as remedies for diseases and play a key role in the human health-care of a vast majority of world's population. World's populations rely on the use of traditional medicine, which is predominantly based on plant material.[3] Numerous tradionally used plants exhibit pharmacological properties with great potential for therapeutic applications in the treatment of central nervous system disorders, such as anxiety disorders [4]. Also because of the increasing desire of people to use herbal medicines in this study been try to anti-anxiety effect of the plant.*vitis vinifera* is a perennial woody plant its leaves are consumed in some in traditional foods use in various food. [5] From the different parts of this plant, in particular from the fruits, several preparations used in folk medicine have been derived. More recently, procyanidins have been demonstrated to be among the most interesting antioxidant agents from Plant Kingdom, and are considered for the preventive therapy of chronic degenerative diseases and the modulation of skin unattractiveness linked to the aging process. It is also used as a nervine tonic . The chemical analysis has shown the presence of procyanidins, anthrocyanins, flavanoids, hydroxylcinnamic acid derivatives, triterpenes, sterols, tannins, polysaccharides, monosaccharides,and non alkaloid nitrogen containing compounds.[6]

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 5, Issue 5 , May 2018

II. MATERIALS AND METHODS

A.Drugs and chemical used: Diazepam , Ranbaxy laboratories . 1% tween solution prepared. All the other chemicals used were of analytical grade and purchased from commercial sources. Other chemicals used for extraction purpose and phytochemical tests were of laboratory grade.

B.Collection and authentication of plants: The leaves of the plant were collected from the Balaji nursery, jagatpura, jaipur district, Rajasthan state, india in month of march 2009. The identity of the collected plant was confirmed by P.J.Parmar, joint Director in Botanical survey of india (BSI) Jodhapur (rajasthan, india) the herbarium of the plants was deposited in the BSI against voucher specimen NO. JNU/JPR/PC/JS-1.

C.Preparation of plant extract: The leaves of plant were washed, shade dried and powdered. The powdered material was defatted with petroleum ether and extracted with methanol by cold maceration process. The extract was concentrated at reduced pressure and temperature in a rotary evaporator. Methanolic extract was tested for presence of secondary metabolites by different phytochemical tests.

D.Preliminary Phytochemical Screening: The methanol extract of vitis vinifera was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, proteins and phenolic compounds. (7-8).

General procedure of qualitative chemical tests

S. NO.	Chemical tests	Observation	Inference
1. A.)	**Test for Carbohydrates** Molisch's test (General Test) To the 2 to 3 ml extract, few drops of α-Napthol solution in alcohol was added followed by addition of conc.H2SO4 from the sides of the test tube.	Violet ring is formed at the junction of two liquids	Presence of Carbohydrates
B.)	Fehling's Test 1 ml. of Fehling's A and 1 ml Fehling's B was mixed in the test tube. Equal volume of extract was added and heated in the boiling water bath for 5-10 min.	First yellow, then brick red precipitate is Observed	Presence of reducing Sugars
C.)	Benedict's Test Equal volume of Benedict's reagent and extract in the test tube was added and heated in a boiling water bath.	Solution appears green, yellow or red	Presence of Reducing sugars
D.)	Barfoed's Test Equal volume of Barfoed's reagent and extract was mixed and heated for 1-2 min. in boiling water and cool.	Red precipitate is observed	Presence of Monosaccharides
2. A.)	**Test for Proteins and aminoacids** Biuret Test To 3 ml of extract 4% NaOH and few drops of 1% CuSO4 Solution was added.	Violet or Pink colour develops	Presence of Proteins
B.)	Ninhydrin Test 3 ml extract and 3 drops of Ninhydrin solution was heated in boiling water bath for 10 min.	Purple or bluish colour appears	Presence of Amino acids
3.	**Test for Fats and Fixed Oils** A small quantity of extract was pressed between filter papers.	Oil stains on the paper	Presence of fixed Oils
4.	**Test For Steroid** Liebermann-Burchard reaction 2 ml of the extract was mixed with chloroform	Purple ring with acid solution turning green	Presence of Steroid

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

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	and 1-2 ml acetic anhydride and 2 drops of Conc. H_2SO_4 was added through the sides of the test tube.		
5. A.)	**Test For Glycosides** Cardiac Glycosides Keller Kiliani test To 2 ml extract, glacial acetic acid, one drop of $FeCl_3$ and Conc. H_2SO_4 was added	Reddish brown layer appears at the junction of two liquids	Presence of Cardiac Glycosides
B)	Legal's Test: The extracts were treated with sodium nitroprusside in pyri-dine and methanolic alkali. The formation of	pink to red color	Presence of cardiac glycosides.
C)	Anthraquinone glycosides Borntrager's test To the extract dil. H_2SO_4 was added. The mixture was boiled and then filtered. To the cold filtrate, equal volume of benzene or chloroform was added. It was then shaked well. The organic solvent was separated and ammonia was added.	Ammoniacal layer turns pink or red	Presence of Anthraquinone glycosides
D)	Saponin glycoside Foam test The drug powder or the extract was shaken vigorously with water.	Persistent foam is observed	Presence of Saponin glycosides
6.	**Test for Phenolic Compounds and tannins** To the extract $FeCl_3$ was added.	Deep blue-black colour formed	Presence of Phenolic Compounds
7.	**Test for Flavonoids** Shinoda Test To the extract, 0.5g of magnesium turnings and few drops of Conc.HCl were added from the sides of the test tube.	Pink colour observed	Presence of Flavonoids
8.	**Test for Alkaloids** Dragendroff's test To the filtrate few drops of dragendroff's reagent was added.	Orange brown ppt. is formed.	Presence of Alkaloids.

E.Experimental animals:

Wistar albino rates of either sex (150-200 gm) were taken for study. They were housed in polypropylene cages in air- conditioned area at at 25±2 ºC with 12/12 h light/dark cycle. All animals had free access to standard pellet diet (Mahavir industries, Delhi) and clean water *ad libitum*. The norms for Good Laboratory Practice (GLP) were followed for care of laboratory animals. The present studies were duly approved by IAEC (Institutional Animal Ethical Committee clearance) 002/2009/IAEC/jnu.

F.Acute toxicity test:

Acute oral toxicity study for the test extract of the plant was carried out using OECD/OCED guideline 425. The test procedure minimizes the number of animals required to estimate the
oral acute toxicity. The observation of signs of toxicity and can also be used to identify chemicals that are have low toxicity. Healthy, young adult albino Wistar rats of either sex (200 -250 g) were used for this study. Animals should be fasted prior to dosing (food but not water should be withheld Overnight). The fasted body weight of each animal is determined and the dose is calculated according to the body weight.[9]

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G.Experimental Design

Anxiolytic activity

To perform this activity 30 overnight starved wistar albino rats (either sex) of 150-200gm body weight. The rats were divided five groups (n=6). Drugs/ vehicle were administered to the animals 60min prior to study.

Group I: Negative control, administer (saline 10 ml/kg of 1% tween) orally.
Group II: Positive control and receive standard drug diazepam (2.5 mg/kg).
Group III: Receive MEVV (100 mg/kg) orally
Group IV: Receive MEVV (200 mg/kg) orally
Group V: Receive MEVV (400 mg/kg) orally

Procedure:

1)Elevated plus-maze test [EPM]

The maze had two arms, 50 × 10cm, crossed with two closed arms having same dimension but having 40cm high walls. The arms were connected with a central square, 10 ×10cm giving the apparatus shape of a plus sign. The maze was kept in a dimly-lit room and elevated 60cm from the ground. after the drug treatment individual rats were placed in the individually in centre of the maze, facing an enclosed arm. There after number of entries and time spent on the open and closed arms were recorded during the next 5 minutes. An arm entry was defined when all four paws of the rat were in the arm. Each rt was assessed individually 30 min after the treatment. [10-11].

2)Open-field test [OFT]

In this test the open field was prepared by using plywood and consisted of squares [65×65cm]. The apparatus was painted black except 5mm thick white lines which divided the floor into 16 squares. Open field was lighted by a 40 watts bulb focusing into the field from a height of about 100cm. the entire room except the open field was kept dark during the experiment. Each animal was centrally placed in the test apparatus for 5 minutes and the following behavioral aspects were noted i.e Ambulation, Rearings, Self grooming, Activity in centre, and Fecal droppings[12].

III.RESULTS

A.Phytochemical screening: The methanol extract of vitis vinifera was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, proteins and phenolic compounds..

B.Acute toxicity test: Acute toxicity studies revealed that *V. vinifera* extract did not produce any toxic symptoms when administered(2000mg/kg) orally to rats.

C.*Anxiolytic activity*

1)Elevated plus maze behavior The extract treated rats exhibited dose dependent significant increase in time spent in open arms, entries made in open arms and significant decrease in time spent in enclosed arms and entries in enclosed arms comparing to control rats. The results obtained by open/closed time and entries ratios also indicated significant anxiolysis in rats by the methnolic extract of *vitis vinifera* (*MEVV*) caused more anxiolysis. [Table 1]

2)Open field exploratory behavior The doses of methnolic extract of *vitis vinifera* (*MEVV)* on rats showed a significant increase in open field ambulation, rearings, self grooming and activity in centre in comparison to vehicle treated rats evidencing significant anxiolytic activity with the standard. [Table 2]

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

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[Table 1]

Effect of *MEVV* in the open and enclosed arms of elevated pluse maze.

Treatment Mg/kg	Time spent in open arm (sec)	Time spent in enclosed arm (sec)	Entries into open arm	Entries into enclosed arm
Tween 10 mL/kg	30.16±0.70	142.33±1.40	4.67±0.71	10.87±2.41
MEVV 100	47±2.3*	132.34±5.65*	9.83±0.94*	10.9±1.33*
MEVV 200	82.17±1.01*	129±9.22*	12.34±1.11*	9.16±2.14*
MEVV 400	91.17±0.87**	115.5±8.81**	19.67±1.11**	6.24±1.07**
Diazepam 2.5	119.16±0.79**	106.83±1.24**	21. 17±2.15**	5.34±1.11**

Value are expressed as mean ± S.E.M. (n=6). Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett"s test. *P<0.05 (significant); **P<0.01(significant) when compare to control rats.

[Table 2]

Open field exploratory behavior

Effect of MEVV and Diazepam on open field exploratory behaviour in rats.

Treatment Mg/kg	Ambulation	Rearings	Self groomings	Activity in centre
Tween 10 mL/kg	46.28±3.20	8.65±1.94	6.54±1.67	1.36±2.11
MEVV 100	68. 01±12.68*	9.8±1.68*	7.12±1.86*	1.96±1.72*
MEVV 200	78.09±5.12*	10.2±2.08*	7.98±1.72*	2.65±0.75*
MEVV 400	82.34±4.12**	12.10±2.01**	8.12±1.5**	4.62±2.88**
Diazepam 2.5	84.66±2.55**	17.16±1.50**	10.86±2.16**	6.12±1.34**

Value are expressed as mean ± S.E.M. (n=6). Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett"s test. *P<0.05 (significant); **P<0.01(significant) when compare to control rats.

IV.DISCUSSION

Preliminary phytochemical investigation the methanol extract of vitis vinifera was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, proteins and phenolic compounds.. In the therapy of anxiety disorder or acute anxiety symptoms, a combination of therapeutic interventions is mostly indicated. Beside a psychotherapeutic approach, anxiolytics are a part of treatment of anxiety.[13] Benzodiazepines are the most widely prescribed 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 [14]. Other

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 5, Issue 5 , May 2018

anti-anxiety medications include antidepressants, buspirone and β -blockers which though effective in many cases, also possess side effects like nausea, light headedness, dizziness, headache, dry mouth, constipation, diarrhea, etc. Self-administration of herbal medicines was among the most popular of alternative therapies, there is considerable interest in the development of new anxiolytics, new therapies for the treatment of anxiety disorders are necessary, and the study of medicinal plants could provide new therapeutic options [15]. The Elevated Plus-maze is a well-established animal model and is currently the first choice test for anxiolytic drugs. It is based on the natural conflict between the drive to explore a new environment and the tendency to avoid potentially dangerous area. In the present study we used the EPM model of anxiety to evaluate the anxiolytic effects of methnolic extract of *vitis vinifera* (*MEVV)* As expected, diazepam produced significant increase in time spent and number of entries into open arms and at the same time showing decreased number of entries and time spent in the closed arm.Therefore, the behavioral alterations induced by the extractsin the EPM are consistent with an anxiolytic effect, similar to that of diazepam. In the this study, we found that methnolic extract of *vitis vinifera* (*MEVV) have* anxiolytic activity (**P* <0.05, ***P* <0.01) in EPM. The different dose of methnolic extract of *vitis vinifera* (*MEVV)* on rats showed a significant increase in open field ambulation, rearings, self grooming and activity in centre in comparison to vehicle treated rats evidencing significant anxiolytic activity with the standard. The results clearly guided us that the methnolic extract of *vitis vinifera* (*MEVV)* are the first choice of interest for further studies although all the extracts have the anxiolytic potential. Further investigations are required to identify the active constituents of the *vitis vinifera* responsible for the anxiolytic effects. The results obtained from this study support the use of this important medicinal plant in the Indian traditional medicine for the management of nervous and cerebral disorders including anxiety. Further studies are in progress in our laboratory to isolate and identify the components responsible for anxiolytic activity and the mechanism of action involved. Results will cover a way for the isolation of bioactive principles and new drug search for anxiety.[16]

V.CONCLUSION

In our present, we revealed the antianxiety activity of methnolic extract of *vitis vinifera* (*MEVV*) at 100, 200 & 400 mg/kg dose level. The results were comparable to that of standard and control group. Further work is needed for the evaluation of isolated compounds activities.

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