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PHARMACOGNOSTICAL AND PHARMACOLOGICAL EVALUATION OF CITRUS RETICULATABLANCO FOR ITS ANXIOLYTIC ACTIVITY

Chavan G. M[1], Dr. Shailendra Sharma[2]
Department of Pharmaceutical Science, Jodhpur National University, Jodhpur, India.

Abstract: ***The objective of this study was to evaluate anxiolytic activity of aqueous, ethanol, chloroform extracts of fruits of citrus reticulatablanco. The investigation was done using elevated plus maze and light and dark exploration test in mice. Anxiolytic activity was assessed by measuring the time spend in open/closed arms in elevated plus maze and measuring time spent in light/dark compartments in light/dark exploration test for the period of five minutes. Albino Swiss mice of either sex weighing 20-25g received various extracts of test drug with different doses orally. Diazepam (2 mg/kg) was used as a reference standard 30 minutes before the activity. The alcoholic & chloroform extracts test drug exhibited significant (P<0.01) anxiolytic activity at dose 400 mg/kg in elevated plus maze as well as light/dark exploration test.***

KEY WORDS: citrus reticulatablanco, Diazepam, elevated plus maze, light and dark exploration test.

I. INTRODUCTION

Anxiety is psychological disorder characterized by persistent and disproportionate fear unrelated to any genuine risk. Apart from few chemical remedies available like benzodiazepines and serotonin modulators, not much options at hand that could safely and effectively alleviate anxiety. There are so many adverse effects produced by allopathic drugs and patents may produce sedation, light-headness, cognitive impairment, sexual dysfunction and some time may produce drug dependence.(K.D.Tripathi et al, 2009) It was revealed that citrus senesis has demonstrated anxiolytic activity which is other plant of the same family having similar genus; whether fruits of these plants possesses the same activity or not is planned to evaluate. Citrus aurantium L., commonly known as sour orange, is used in Brazilian folk medicine and other countries to treat anxiety, insomnia, and as an anticonvulsant suggesting depressive action upon the central nervous system, among other properties. (Maria Isabel Roth et al, 2002)

II. MATERIAL AND METHODS:

Plants material and extract preparation

The plant has been collected in month of March-April from Paratwada region of Dist-Amarawati (M.H.) and authentication has been obtained from Dept. of Botany, PSGVPM Arts & Science College, Shahada, DistNandurbar (M.H.). Voucher specimens and herbarium sheet is kept in the institute for further references. In one group freshly prepared juice is taken for the investigation whereas in another groups extraction has been done by using successive solvent extraction scheme because we didn't know which constituent of the fruit is responsible to claim anxiolytic activity as it provides all chemicals present in the plant in fraction form. The air dried powdered plant material i.e. whole fruit (50-100 g) was extracted successively with the following solvents of increasing polarity in a Soxhlet extractor: 1 Chloroform 2. Ethanol (K. R. Khandelwal, 2011) The reason behind performing extraction of fruit part is that it contains number of active constituents. (K. M. Nadkarni, 1976) The extracts were tested for the anxiolytic activity by two different methods. The extracts were administered orally in the concentration 100, 200 and 400 mg/kg in elevated plus maze as well as light and dark exploration paradigm method.

Animal

Albino Swiss mice of either sex weighing 20-25 g were housed in group of six under standard laboratory conditions of temperature and 12/12 hour light and dark cycle. They had free access to food and water. All the experiments were conducted at the time from 9.00 to 15.00 hours. Mice were maintained in plastic cages. Animals were deprived of food but not water 12 hour before the experiments. The animals were acclimatized to laboratory condition for not less than 10 days. The employed experimental protocols were as per the ethical principles and guidelines and approved by institutional animal ethical committee constituted for the purpose of control and supervision of experimental animals by ministry of Environmental and Forests, Government of India, New Delhi.

Assessment of anxiolytic activity:

1] Elevated plus maze test: -

Evaluation of anxiolytic activity of extracts of citrus reticulatablanco in mice were assessed by using elevated plus maze (EPM) as described by Pellow et al., 1985. The elevated plus maze for mice (Lister, R. G. 1987) consist of two open arms (37X5) (File S F, Pillow S, 1985) and two enclosed arms (37X5X12) with 12 cm high wall arranged so that the arms of the same type were opposite to each other. The arms were connected with a central square of 5X5 cm. The wooden apparatus was elevated to a height of 25 cm. above the floor. The mice in the groups of six treated with drugs listed earlier and vehicle were placed individually in the center of the EPM facing towards open arm and time spent in open and enclosed arm was recorded for 5 minutes. The ratio of time spent in open verses enclosed arm was calculated. Each animal should be used only once and the test should be carried out during a fixed time of the day. The rationale is that the open arms are more fear-provoking and that the ratio of either time spent on open: closed arms or

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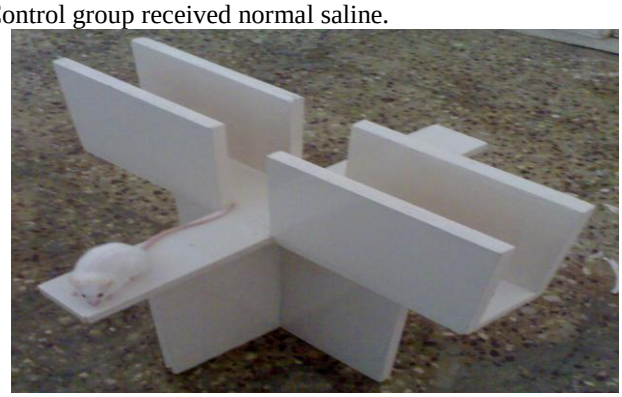
entries into open-closed arms reflect the relative "safety" of closed arms compared with the relative "fearfulness" of open arms. Anxiolytics would be expected to increase the proportion of entries into and time spent on open arms. The EPM was cleaned with hydrogen peroxide after each trial. Control group received normal saline.



2] Light and dark exploration test: -
This model (Crawley J N, 1981) is based on natural aversion of mice to brightly light places. The apparatus consists of two compartments box, one dark and the other brightly light. Since anxiolytic reduces the natural aversion to light compartment. They spend more time in light compartment. Anxiogenic agents, on contrary, reduce this time and spend more time in the dark compartment. A typical apparatus consists of a wooden box (45X27X27 cm). The box is open topped and dimly illuminated (10 W white bulb). Naïve mice were placed individually in the center of the light compartment and observed for the next 5 minutes for the number of crossing between two compartments and time spent in the Light and dark compartment. The animal received extracts of citrus reticulatablanco 30 minute before test. Diazepam in dose of (2mg/kg) was used as a reference standard.
Statistical analysis: -
All results are presented as mean ± s.e.m. Analysis of variance was followed by Dunnett's test as post-hoc test. Results would be determined by value of P.

III. RESULTS

The elevated plus maze test (TABLE 1): Oral administration of extract of fruits of citrus reticulatablanco increases number of entries as well as time spent in open arm at highest dose 400 mg/kg.

TABLE 1: Effect of various extracts of citrus reticulatablanco on Elevated plus maze in mice

Group	Treatment	Average time spent in open arms				
		Extracts			Control	
		Chloroform	Ethanol	Aqueous	Negative	Positive
1	Vehicle	-	-	-	50.66 ±1.25	
2	Diazepam	-	-	-		153.95 ±1.45
3	100mg/kg	114.27 ±1.23	125.5± 1.25	90.31 ±1.86		
4	200mg/kg	118.89 ±2.09	130.33 ±1.83	96.52 ±1.77		
5	400mg/kg	121.45 ±1.66	138.16 ±1.10	99.89 ±1.91		

The values are reported as Mean±SEM for the number of mice shown in parenthesis* P<0.001 verses control(Analysis of variance and Dunnett's test as the post hoc test). 400 mg/kg of ethanolic extract of citrus reticulatablanco increases time spent in open arm by 272% (138.16±1.10) as compare to control (50.66±1.25); the chloroform extract and aqueous extract appear to be devoid of anti anxiety activity since there P value are insignificant. Diazepam (2 mg/kg) orally used as a positive control, increases time spent by 303% (153.95±1.45) as compare to control (50.66±1.25).

TABLE 2: Effect of various extracts of citrus reticulatablanco on Light and Dark Exploration paradigm in mice

Group	Treatment	Average time spent in Light compartment				
		Extracts			Control	
		Chloroform	Ethanol	Aqueous	Negative	Positive
1	Vehicle	-	-	-	36.66 ±1.76	-
2	Diazepam	-	-	-	-	153.95 ±1.45
3	100mg/kg	89.21± 1.38	98.33± 1.62	81.51 ±1.86		
4	200mg/kg	100.29 ±1.59	117.66 ±1.33	86.18 ±1.77		
5	400mg/kg	111.88 ±1.72	126.76 ±1.63	95.19 ±1.91		

The values are reported as Mean±SEM for the number of mice shown in parenthesis* P<0.001 verses control(Analysis of variance and Dunnett's test as the post hoc test).

Light and Dark exploration test (TABLE 2)
Diazepam (2 mg/kg) treated mice increases the time spent into the light compartment {F(4,19)= 153.95±1.45, P<0.01} by 419%. The acute administration of ethanolic extract of citrus reticulatablanco increases time spent at highest dose 400 mg/kg after oral administration by 345% (126.76±1.63) as compare to control (36.66±1.76).

IV. DISCUSSION

The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Anxiolytic agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms (Kumar S. et al, 2005). In this study the percentage of time spent in open arms of the elevated plus maze paradigm and percentage of time spent in light compartment of light and dark exploration paradigm were taken as a measure of anxiety. Benzodiazepines show a strong anxiolytic profile (H.J.Little, 1991), hence diazepam was used as a positive

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control. The ethanolic extract of citrus reticulatablanco (400 mg/kg), markedly increased the percentage of average time spent by the animal in the open arms. The anxiolytic effect of plant extract was more prominent at 400 mg/kg and doses lower than this did not show a consistent anxiolytic effects. The anxiolytic effect of ethanolic extract of citrus reticulatablanco may be related to their flavonoid content. In addition extract of citrus reticulatablanco may interact with receptors of various neurotransmitters.

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