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Original Article

CNS DEPRESSANT, SEDATIVE AND ANXIOLYTIC ACTIVITY OF ETHANOLIC EXTRACT OF FRUIT OF PIPER CHABA REVEALED AFTER NEUROPHARMACOLOGICAL SCREENING

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ABSTRACT

Objective: Different ailments can be treated by multiple approaches. Since ancient civilization plant material have been used successfully for treating diseases. Plants contain active ingredients which are beneficial for treatment of multiple diseases. The purpose of the study was to evaluate the neuropharmacological effects of ethanolic extract of fruit of *Piper chaba.*

Methods: *Piper chaba's* ethanolic extract was diluted in DMSO and administered orally at 300mg/kg according to weight of mice for 21 days. A number of tests were then performed to evaluate CNS activity.

Results: The results showed reduced number of cage crossing, head dips, decreased central and peripheral square crossing, reduced struggling time in forced swim test and decreased time spends in light and dark test.

Conclusion: From our results it is concluded that after acute dosing ethanolic extract of fruit of *Piper chaba* possesses CNS depressant, mild anxiolytic and sedative effect.

Keywords: *Piper chaba*, Piperaceae, Anxiolytic, Sedative, Depressant, GABA (Gamma amino butyric acid), DMSO (Dimethyl Sulfoxide).

INTRODUCTION

Phytochemicals are synthesized by plants which are chemical substances used in ailments of different diseases [1]. Antioxidants are part of phytochemicals which possesses biological activities such as reduction in risk of cancer, aging etc [2]. Plants had been used for healthcare and medicinal purposes long before it was recorded in history [3]. Studies reveal that the clinical settings in Europe and USA have a very low rate of prescribing herbal medicine but recently herbal medicines have regained their popularity because of the availability of scientific evidence supporting the effectiveness and safety of herbal medicine [4].

Piper is an ecologically and economically important genus in family Piperaceae [5]. Most piper species are herbaceous climbers or vines; some grow as shrubs while others grow as small trees [6]. Family Piperaceae has found the great deal of applications in the traditional pharmacopeias of several cultural groups such as Indian ayurvedic system, Chinese herbal medicine, as well as in Latin, African, American and West Indies medicine [7].

The plant *Piper chaba* grows yearly and is a lasting shrub [8]. A phytochemical analysis was performed on *Piper chaba* hunter. The major components of stem bark are: Piperine, Piperanine, Pipernonaline, Dehydropipernonaline, Piperlonguminine, Retrofractamide B, Guineensine, N-isobutyl-(2E, 4E)-octadecadienamide, N-isobutyl-(2 E, 4 E, 14 Z)-eicosatrienamide, Lignan [9] and alkaloids such as piperamine 2, 4-decadienoic acid piperidide, kusunokinin and pellitorine [10]. A unique piperine dimer CHABAMIDE has also been isolated from stem bark [11].

Some alkamides are reported to be present in the root of *Piper chaba* such as: Piperine, Piplartine, Piperlonguminine, Sylvatine and β-sitosterol [12]. The fruit oil of *Piper chaba* contains caryophyllene oxide, β-caryophyllene, few monoterpenes hydrocarbons, high amount of aliphatic hydrocarbons and moderate content of sesquiterpenes [13].

Piper chaba has been used in traditional medicine as carminative, stimulant, anti-hypertensive, muscle relaxant [14]. Stem is useful in diarrhea, arthritis and rheumatic pains [15]. The fruit of *Piper chaba* has shown a lot of potential in traditional medicine. It is used as an anti-flatulent, gastro-protective, appetizing property, as an expectorant, anti-tussive, anti-fungal agent. It also possesses cholesterol lowering properties [16].

The activities exhibited by *Piper chaba* fruits are anti-inflammation, chemoprevention, hepatoprotection, antiangiogenesis, adipogenesis and immunomodulation [17]. In Ayurveda its stem has shown diuretic, anti-inflammatory, analgesic, antidiarrheal and CNS depressant activity in mice [18]. The methanol and ethanol extract of stem bark at 125mg, 250mg and 500mg/kg dose dependently, decreased carrageenan induced paw edema in rat and increased pentobarbitone induced sleeping time in mice [19]. The aim of an above study is to determine neuropharmacological effects of ethanolic extract of fruit of *Piper chaba* after acute dosing in order to gain knowledge about its efficacy and toxicity.

MATERIALS AND METHODS

Plant Collection

The plant *Piper chaba* was provided by Dr. Iqbal Azhar Department of Pharmacognosy University of Karachi.

Extraction of plant material

Inorder to reduce the microbial load the plant was first washed with water. The fruit of *Piper chaba* was cut into small pieces and dried at 50°C, and then they were powdered and extracted. Next this powdered material was macerated with 95% ethanol for 3 days. It was then filtered and reduced to dryness under pressure. The process of maceration was repeated twice and then dried using evaporator.

Selection of animal's

For screening of CNS parameters 20-26 gm albino mice of either sex bred at animal house of the Department of Pharmacology, University of Karachi was used. The mice were given a standard diet ad libitum and water for 21 days. The environmental conditions were kept constant i. e 23+2°C.[20] All animals were equally divided into three groups, one group served as control, second as standard (lorazepam) and third as treated with ethanolic extract of *Piper chaba*. Animals were handled as per specifications provided in

Helsinki Resolution 1964 and study was approved by our Board of advanced studies and research vides Resol. NO.10 (6) dated 26-09-2012 & 16-10-2012.

Dosing protocol

The dosing of *Piper chaba* was done on the daily basis. Ethanolic extract of fruit of *Piper chaba* was taken and the dose was taken as: *Piper chaba* 300mg/kg (for acute dosing) [21] so dose adjusted according to weight of mice in milligrams. Standard solution was prepared of 250mg/10 ml in DMSO and dose was given orally in milliliter (ml) by the serial dilution method.

Control mice were given similar milliliter (ml) of DMSO.

Standard drug used was Lorazepam 2mg/60kg that means 0.3 mg/kg. This dose was adjusted according to weight of mice in milligrams. Stock solution was prepared 12mg/60 ml in DMSO and dose was administered by the serial dilution method orally.

CNS screening test

Cage crossing test

For monitoring exploratory behavior of mice transparent, plexiglass cage (26×26×26 cm) with saw dust covered floor was used. The mice of all 3 groups were placed in an apparatus for 5 minutes to get them customized with apparatus. After they got acquainted with the setting, the numbers of cage crossings were counted for 5 minutes [22].

Head dip test

Another exploratory behavioral test used for evaluation of different anxiety related activity in rodents is hole board or head dip test. The apparatus consists of an enclosed wooden rectangular box (35 cm×45 cm×45 cm).

The holes are 2.5 cm in diameter and found in all walls. [23] The mice that were not customized with the apparatus were placed in the central area and allowed to freely explore for 5 minutes. The number of times the mouse stuck out its snout was noted [24].

Forced swim test

It measures the antidepressant effects of drugs. It is maintained at 22-25°C and consists of the cylindrical container made of glass containing 8 cm water [42] the mouse is placed in such way, that feet of animal do not touch the bottom.

The test is based on the assumption that the animal will swim actively inorder to escape from stressful stimuli. Animal shows state of despair when it stops swimming and floats on surface. Normally it is performed for 5 minutes in mice. The time at which immobility is achieved is recorded [25].

Open field test

Another test to assess emotional behavior in rodents is an open field test. The mice were held gently by the tail and placed in centre of arena in open field. The activity of mice was observed for 10 minutes.

During this experiment, we observed the number of times the mice moved in the centre square using all 4 paws and the number of peripheral squares crossed by mice on all 4 paws.

Light and dark test

This test was used to assess anxiety behavior in rodents. Rodents generally favor dark areas. The instinctive conflict between risk avoidance and exploratory drive is thought to inhibit exploration. The important feature that is observed is the change in willingness to explore the lightened unprotected area [26].

Statiatical analysis

By taking mean of all the values, they are compared with means of control and the standard drug and by student significance t-test the significance of difference between means is determined. A value of $p< 0.05$ is considered significant, $p< 0.001$ as more significant and $p< 0.0001$ as highly significant. By Alcarz and Jimenez method all statistical procedures are performed [27].

RESULTS AND DISCUSSION

Herbal preparations have been used since ancient civilizations as the source of medicinal agents because of their therapeutic efficacy, safety and low cost. *Piper chaba* a member of family piperaceae family is commonly found in tropical areas and possesses extensive pharmacological uses.

Table 1.1 shows that when 300mg of *Piper chaba* was administered orally once daily. Significant decrease in cage crossing activity was observed after 7,14 and 21 days. From the above we can conclude that *Piper chaba* possesses CNS depressant effects. Previous studies show that locomotor activity is controlled by peripheral signals from spinal cord and brain area which plays a role in controlling movement and posture is the cerebellum [28].

Decreased locomotor activity indicates depressed CNS activity. It is known that GABA is major inhibitory neurotransmitter in CNS. The inhibitory effect is usually mediated by binding to $GABA_A$ receptor [29]. The mechanism of action of different anxiolytic drugs is by binding to $GABA_A$ receptor which causes chloride ions influx leading to hyperpolarization which reduces firing rate of critical neurons in brain [30]. Piperine is the main constituent of Piper species which possess CNS depressant effect [31]. Exact mechanism of action is not known but it can be postulated that it acts on $GABA_A$ receptor; further work can be done on *Piperchaba* to evaluate its mechanism of action leading to CNS depression.

Above postulation is further confirmed by the Open field test. Table 1.2 shows the number of central square crosses was decreased after 7, 14 and 21 day dosing of *Piper chaba.* Open field test is conducted to observe the exploratory behaviour, locomotor activity and anxiety in rodents. [32] Central square crossing is done to evaluate anxiety and exploratory behavior [33]. Our results showed reduced number of central square crossings indicating CNS depressant effect.

Table 1.3 shows the number of peripheral square crosses were decreased after 7, 14 and 21 day dosing of *Piper chaba*. Peripheral square crossing is indicative of thigmotaxis a phenomenon in which due to fear and anxiety the mouse tries to stay near proximity of wall's [34]. The results show decreased peripheral crossings indicating CNS depressant and anxiolytic activity. The increased frequency of urination and defecation has also been described as a marker of anxiety [35]. Our study indicates that after 21 day dosing of extract the urination and defecation frequency did not increase showing the extracts relieve anxiety.

Table 2 shows there was a significant decrease in head dip activity by *Piper chaba* on acute dosing. Due to fear and neophobia animal tries to escape on initial exposure to the apparatus [36]. Elevated levels of corticosteroids in adult rats following first exposure to apparatus further confirms stressful condition of animal [37]. If it is assumed that on exposure to apparatus, anxiety develops due to state of fear so decrease in number of dips shows relieve from anxiety or reduced fear [38]. This postulation supports our above results that our extracts possess anxiolytic effect.

Table 1.1: Effect of *Piper chaba* on Exploratory Activity (Cage Crossing)

Effect of *Piper chaba* on Cage Crossing									
				Number of Cage Crosses					
	Day 7			**Day 14**			**Day 21**		
Groups	**Mean ± SD**	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**
Control	45.3±2.31			45.2 ± 1.75			47.1±1.73		
Standard	27.7±1.77			13.9± 1.66			7.8± 1.55		
Piper chaba	35.0±1.63	***0.000	***0.000	21.2± 1.03	***0.000	***0.000	13.6± 1.17	***0.000	***0.000

Table 1.2: Effect of *Piper chaba* on exploratory activity (Central Square Crossing)

Effect of *Piper chaba* on Central square crosses in Open field test									
Number of Central square crosses									
	Day 7			Day 14			Day 21		
Groups	**Mean ± SD**	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**
Control	33.4±1.17			32.1 ± 1.45			30.7±1.06		
Standard	17.2±1.32			12.3± 1.37			5.0± 1.49		
Piper chaba	19.7±1.16	***0.000	***0.000	13.9± 1.37	***0.000	*0.046	11.2± 1.03	***0.000	***0.000

Table 1.3: Effect of *Piper chaba* on Exploratory activity (Peripheral Square Crosses)

Effect of *Piper chaba* on Peripheral square crosses in Open field test									
Number of Peripheral square crosses									
	Day 7			Day 14			Day 21		
Groups	**Mean ± SD**	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**
Control	137±1.25			131.6 ± 1.78			138.7±1.49		
Standard	167.1±2.6			50.8± 2.94			15.1± 3.07		
Piper chaba	104.7± 1.49	***0.000	***0.000	84.4± 1.43	***0.000	***0.000	27.0± 1.49	***0.000	***0.000

Table 2: Effect of *Piper chaba* on Anxiolytic activity (Head Dip Test)

Effect of *Piper chaba* on Head Dip Activity									
Number of Head Dips									
Groups	Day 7			Day 14			Day 21		
	Mean ± SD	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**
Control	34.5±1.58			33.8 ± 1.62			32.7±2.0		
Standard	34.4±1.17			17.9± 1.66			12.8± 1.75		
Piper chaba	21.9±1.79	***0.000	***0.000	17.0± 1.49	***0.000	***0.000	16.1± 1.6	***0.000	***0.000

Table 2.1: Effect of *Piper chaba* on Anxiolytic activity (Light and Dark Test)

Effect of *Piper chaba* on Light and Dark Activity									
Time spent in light box (sec)									
Groups	Day 7			Day 14			Day 21		
	Mean ± SD	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**
Control	473.2±2.62			487± 1.49			485.1±1.45		
Standard	175.8±3.33			124± 3.4			16.1± 3.48		
Piper chaba	202.6± 1.9	***0.000	***0.000	182.5± 1.84	***0.000	***0.000	69.5± 1.08	***0.000	***0.000

Table 3: Effect of *Piper chaba* on Anti-Depressant Activity.

Effect of *Piper chaba* on Forced Swimming Test									
Struggling Time (sec)									
Groups	Day 7			Day 14			Day 21		
	Mean ± SD	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**	**Mean ± SD**	**P(control)**	**P(STD)**
Control	92.7±2.0			84.8 ± 1.03			95.8±1.03		
Standard	60.1±1.66			30.10± 1.66			12.1± 1.52		
Piper chaba	53.0±2.45	***0.000	***0.000	38.5± 1.58	***0.000	***0.000	28.8± 1.48	***0.000	***0.000

Values are mean ± S. D, N=10= number of animals, *p<0.05 = significant, ***p<0.0001 = highly significant , Following t-test and ANOVA df (2, 29)

Anxiolytic or anxiogenic effects of drugs are also determined by light and dark test. In this the exploratory response of rodents is observed when exposed to stressors as light and changed environment as well as it focuses on instinctive nature of rodents to repel brightly illuminated area. [39] Research studies have shown that an animal who is stressesd or in fear or has anxiety tends to stay for prolonged period in darker area. He will not prefer to move and explore about the white box and peaking between two boxes will also be low. On the other hand anxiolytic drugs increase the number of transitions and the time spend in the white area [40].

In acute dosing of *Piper chaba* we observed decreased number of transitions showing its CNS depressant effects.

Table 3 shows that in *Piper chaba* treated mice the immobility state was achieved within seconds. Forced swim test is not only an indicator of anti-depressant effect of drugs but it is also used as an indicator of depression in rodents [41.]

when mice become immobile after period of vigorous activity it represents depressive state.[42] A pathological complex of psychological, neuroendocrine and somatic symptoms is clinically represented as Depression[43]. This confirms CNS depressant activity of extract. $GABA_A$ α_1 isoform mediates sedative and ataxic effects too [44]. The above results show reduced locomotor and sedative effect which is similar to effects produced by standard drug Lorazepam.

CONCLUSION

From our research study we can conclude that after neuro pharmacological screening of ethanolic extract of fruit of *Piper chaba* after acute dosing we found that it possesses CNS depressant, Sedative and mild anxiolytic effect. Further studies can be conducted to evaluate its effect on specific regions of brain and neurotransmitters.

CONFLICT OF INTERESTS

None to declare.

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