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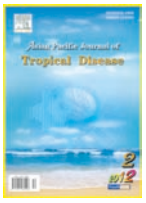

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Anxiolytic effect of *Oxalis corniculata* (Oxalidaceae) in mice

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ABSTRACT

Objective: To investigate the anxiolytic effect of *Oxalis corniculata* in mice was examined. **Methods:** The open field test, elevated plus maze test and anti-fighting test were used to assess the anxiolytic activity of ethanolic extract of *Oxalis corniculata*. Diazepam 1 mg/kg served as a standard anxiolytic drug, administered intraperitoneally. **Results:** Ethanolic extract of *Oxalis corniculata* (100 and 300 mg/kg) produced a significant increase in the number of squares crossed (controls = 24.33±3.48), but significantly decreased both the immobility (controls = 47.17±4.29 sec) and fecal pellets (controls = 13.50±0.96 fecal pellets) when compared with control mice in the open-field test; they significantly increased the number of entries (controls = 53.00±2.67 sec) in the open arms, but decreased both the number of entries (controls = 29.33±1.05 entries) and time spent (controls = 166.7±4.30 sec) when compared with the control mice in the closed arms of the elevated plus-maze test. Furthermore, ethanol extract of *Oxalis corniculata* (100 and 300 mg/kg) decreased the fighting episodes significantly (controls = 9.50±0.62 fighting episodes) when compared with control mice. In addition these results were found to be consistent with anxiolytic effect produced by diazepam. **Conclusions:** The results of present study suggest that an ethanolic extract of *Oxalis corniculata* may possess anxiolytic activity and provide scientific evidence for its traditional claim.

1. Introduction

Oxalis plant is one of the most demandable plant species in India that are having several gray areas which are the focus to the future researchers. It is a sub-tropical plant and originated from India[1]. The plant having most diversed genus and consist of about 900 species[2]. *Oxalis corniculata* Linn. is commonly known as creeping woodsorrel, belongs to the family Oxalidaceae. It is a somewhat delicate-appearing, low growing, herbaceous plant. It is distributed as a weed in damp shady places, roadsides, plantations, lawns, nearly all regions throughout the warmer parts of India and Ceylon, in the Himalayas up to 8,000 ft- cosmopolitan[1-4]. Traditionally, preparations from various parts of the plant, such as leaves, stem and root are used as remedies for various illnesses[5]. The plant is used as stimulant and tonic; beneficial in chest pain, convulsions, cramps and inflammatory tumor[6]. Fresh juice of plant given in dyspepsia, piles, insomnia, anaemia and tympanitis[7]. Ground leaves are eaten as chutney that acts as blood purifier[1]. It is also used for giddiness, diarrhea and dysentery, juice of leaves applied to open wound relieves pain, paste of ground leaves and raw onions applied to forhead for intense headache[8]. The plant is well known for its medicinal value as a good appetizer and as a remover of Kapa, vata and piles[9]. Reported medicinal activity of this plant are anti-implantation and abortifacient[10], wound healing[11], anti-diarrhoeal[12], hypoglycemic effect[13] and anti-fertility activity[14]. Phytochemical investigations of *Oxalis corniculata* Linn. have revealed the presence of tannins, palmitic acid, a mixture of oleic, linoleic, linolenic and stearic acids[15]. Methanolic and ethanolic extracts of this plant show the presence of carbohydrate, glycosides, phytosterols, phenolic compounds, flavanoids, proteins (12.5%), amino acids and volatile oil[9]. Although several medicinal uses have been reported for *Oxalis corniculata*, but the plant have not been examined for their CNS effects. Hence, in this paper, we investigated the antianxiety effect of ethanolic extract of *Oxalis corniculata* using the open-field, elevated plus-maze and anti-fighting tests.

2. Materials and Methods

2.1. Experimental Animals

Swiss albino mice (18-24 g) were used for the study. The

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inbred colonies of mouse were maintained in the animal house of Siddhartha Institute of Pharmacy, Dehradun, India for experimental purpose. The animals were maintained under controlled conditions of temperature (23±2 ℃), humidity (50±5%) and 12 h light-dark cycle (light on from 06:00 to 18:00 h). All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free assessed to standard pellets as basal diet and water ad libitum. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Siddhartha Institute of Pharmacy, Dehradun, India.

2.2. Plant material

The fresh whole plant of *Oxalis corniculata*, were collected from Medicinal garden of Siddhartha Institute of Pharmacy, Dehradun, India in the month of August, 2010. The plant was identified and authenticated by Dr. S.B. Singh, Scientist, NISCAIR, New Delhi. A voucher specimen (NISCAIR/RHMD/consult/-15-10-10/1625/109) was deposited in the herbarium of NISCAIR, India.

2.3. Extraction

The plant was air dried under shade, pulverized with a mechanical grinder and stored in a closed glass container until the time of extraction. The air-dried pulverized plant (900 g) was extracted with distilled ethanol (90%) by soxhlet method to obtain the ethanol extract of *Oxalis corniculata* (EEOC). The extract was concentrated to a small volume by rotary evaporator apparatus and then dried at room temperature, corresponding to a 7.6% yield. The dried extract was suspended in 1% Tween 80 for animal administration.

During the tests, this compound was given twice daily using an appropriate oral needle for 5 days. The mice were than tested 4 hr after the final administration of the test compound. The animals were divided into four groups, each containing six mice. The groups of mice were assigned to receive one of the following: (i) vehicle (distilled water 0.1 ml/10 g of body weight i.p.); (ii) diazepam (1 mg/kg, i.p.); (iii) EEOC (100 mg/kg, p.o.); (iv) EEOC (300 mg/kg, p.o.); this group pattern was used to assess the anxiety parameters. The doses of EEOC were selected on the basis of previous experiments conducted in our laboratory.

2.3. Phytochemical screening

The phytochemical tests to detect the presence alkaloids, carbohydrates, glycosides, phytosterols, phenolic compounds/tannins, flavonoids, proteins, aminoacids and volatile oils were performed according to the method described by Gupta et al[16]. The tests were based on the visual observation of color change or formation of a precipitate after the addition of specific reagents. The results for the extracts studied are shown in Table 1.

Table 1

Phytochemical screening of ethanolic extract of *Oxalis corniculata*

Phytochemical constituents	Ethanol extract
Alkaloids	-
Glycosides	+
Carbohydrates	+
Tannins	+
Phenolic compounds	+
Phytosterols	+
Fatty acids	-
Flavonoids	+
Amino acids	+
Proteins	+
Volatile oils	+

'+' indicate the presence of compounds; '-' indicates the absence of compounds.

2.4. Screening methods for anxiolytic activity

2.4.1. Open-field test

The open-field test is considered to be an indicator of the emotional state of the test animals. This test was performed at a low noise behavioral room, with subdued light in a quiet room but adequate ventilation for at least 1 hr before the experiment. They were tested by visual observation for anxiolytic activity in the above conducive environment. After oral administration of EEOC (100 and 300 mg/kg), each mouse was put in a corner of the open-field with dimensions of 92 cm x 92 cm with 16 squares division and observed for 15 min in three-5 min periods[17]. During the test period, the number of squares crossed, the time spent in total pellets was recorded. Between tests, the apparatus was thoroughly cleaned.

2.4.2. Elevated plus-maze test

The elevated plus-maze test is a rapid and selective technique. It is capable of detecting anxiolytic and anxiogenic drug effects under identical conditions. The plus-maze is in the shape of a cross or plus with two closed arms, measuring 25 x 5 cm, running along a north-south axis and two open arms (similar dimensions with the closed arms) running east-west. The height of the apparatus is 30 cm and was elevated 25 cm from the floor in a dimly illuminated room.Male mice were placed individually in the center of the maze, facing an enclosed arm and the time spent on the open and closed arm were recorded during the next 5 min[17]. An arm entry was defined as all four feet in the arm. The apparatus was cleaned after each use.

2.4.3. Anti-fighting effect

Pairs of male mice were placed under a glass beaker on a grid constructed of stainless steel rods. Foot shocks of 2-mA intensity were delivered for 3 min and the frequency of fighting episodes was noted. Mice that showed 5 or more fighting episodes were selected for this study. The mice pairs were re-tested after drug treatments and fighting episodes were recorded during the 3 min observation period[18].

2.5. Statistical Evaluation

The data were expressed as mean±S.E.M. Statistical comparisons were performed by one-way ANOVA followed by Tukey's post-test using Graph Pad Prism version 5.0, USA. $P<0.05$ was considered significant.

3. Results

Table 1 shows the effects of distilled water, diazepam and EEOC (100 and 300 mg/kg) on the open-field test in mice. Results showed that mice administered with 0.1 ml/10 g of distilled water and 1 mg/kg of diazepam exhibited crossings of 24.33±3.48 and 45.33±2.01 squares, immobility of 47.17±4.29 and 25.50±3.10 sec, 13.50±0.96 and 3.67±0.49 fecal pellets, respectively, in the open-field test. Results also indicated that EEOC (100 and 300 mg/kg) significantly increased the number of squares crossed, 29.33±2.14 and 36.33±2.50 squares, but also significantly decreased immobility, 40.83 ±3.96 and 32.67±2.51 sec, fecal pellets, 11.00±0.37 and 9.83± 0.48 fecal pellets, when compared with controls ($P<0.05$ and $P<0.01$) in the open-field test.

Table 2 shows the mice administered with distilled water and diazepam made 13.83±0.83 and 32.67±1.48 entries and also spent 53.00±2.67 and 110.3±5.41 sec, respectively, in the open arms of the elevated plus-maze test. In addition EEOC (100 and 300 mg/kg) significantly increased the number of entries, 19.50±1.60 and 21.17±0.60 entries and also time spent, 76.00±3.09 and 100.0±3.06 sec, in the open arms when compared with controls ($P<0.05$, $P<0.01$ and $P<0.001$) in the similar test. In contrast, control and diazepam-treated mice made 29.33±1.05 and 11.33±0.92 entries and spent 166.7 ±4.30 and 66.83±3.67 sec, respectively, in the closed arms of the similar test. In addition, EEOC (100 and 300 mg/kg) significantly decreased both the number of entries, 24.67± 1.22 and 22.17±1.11 entries and time spent, 151.0±4.41and 140.0±3.31 sec, in the closed arms when compared with controls ($P<0.05$ and $P<0.001$) in the elevated plus-maze test.

Table 3 shows that vehicle and diazepam treated mice produced 9.50±0.62 and 2.67±0.33 fighting episodes respectively. Results also indicated that EEOC (100 and 300 mg/kg) significantly inhibited the fighting behavior, 7.00± 0.58 and 4.33±0.42 fighting episodes, when compared with controls ($P<0.05$ and $P<0.001$) in the anti-fighting test.

Table 2

Effects of diazepam and ethanolic extract of *Oxalis corniculata* on the open-field test in mice

Treatment (dose)	squares crossed(n)	Immobility(sec)	Fecal Pellets(n)
Vehicle (0.1 ml/10 g)	24.33±2.71	47.17±2.19	13.50±0.96
Diazepam (1 mg/kg)	45.33±2.01c	25.50±2.16b	3.67±0.49c
EEOC (100 mg/kg)	29.33±2.14	40.83±2.96	11.00±0.37a
EEOC (300 mg/kg)	36.33±2.50a	32.67±2.51a	9.83±0.48b

Values are expressed in mean±SEM, Where n=6

a=*$P<0.05$, b=**$P<0.01$ and c=***$P<0.001$; compared with vehicle treated group

Table 3

Effects of diazepam and ethanolic extract of *Oxalis corniculata* on the elevated plus-maze test

Treatment (dose)	Number of entries (n)		Time spent (sec)	
	Open arms	Closed arms	Open arms	Closed arms
Vehicle (0.1 ml/10 g)	13.83±0.83	29.33±1.05	53.00±2.67	166.7±4.30
Diazepam (1 mg/kg)	32.67±1.48c	11.33±0.92c	110.3±4.41c	66.83±3.67c
EEOC (100 mg/kg)	19.50±1.60a	24.67±1.22a	76.00±3.09b	151.0±4.82a
EEOC (300 mg/kg)	21.17±0.60b	22.17±1.11c	100.0±3.06c	140.0±3.31c

Values are expressed in mean±SEM, Where $n=6$

a=*$P<0.05$, b=**$P<0.01$ and c=***$P<0.001$; compared with vehicle treated group

Table 4

Effects of diazepam and ethanolic extract of *Oxalis corniculata* on the fighting episodes in mice

Treatment (dose)	Fighting episodes (n)
Vehicle (0.1 ml/10 g)	9.50±0.62
Diazepam (1 mg/kg)	2.67±0.33c
EEOC (100 mg/kg)	7.00±0.58a
EEOC (300 mg/kg)	4.33±0.42c

Values are expressed in mean±SEM, Where n=6

a=*$P<0.05$ and c=***$P<0.001$; compared with vehicle treated group

4. Discussion

Various behavioral tests have been used to measure anxiolytic activity. In the open-field test, where animals are taken from their home cage and placed in an unfamiliar environment, normally they showed anxiety and fear by remaining immobile, decreasing ambulation, exploration and freezing, but increasing defecation due to heightened autonomic anxiety. These

Phenomena will be attenuated by classical anxiolytic such

as diazepam that is used in this study but augmented by anxiogenic agents[19].

Likewise, the elevated plus-maze is another widely-used test model. Normally, mice spend most of their time in the closed arms and avoided the open arms (afraid, possibly, of falling off). As such, administration of anxiolytic drugs such as diazepam increase the time spent in the open arms and also increases the mobility as evidenced by the frequency of crossing the transection[20] as compared to the controls. Unlike both the above experimental test models that are dependent upon fear and anxiety, the fighting behavior in paired rodents represents hyper emotionality and inherent aggressive behavior.

The beneficial medicinal effect of plant materials typically results from the combinations of secondary metabolites present in the plant, through additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process. This fact has a basis in the sense that medicinal actions of plants are unique to particular plant species or groups, consistent with the concept that combinations of secondary metabolites in a particular plant are often taxonomically distinct phytochemical analysis on this plant revealed the presence of glycosides, carbohydrates, tannins, phenolic compounds, phytosterols, flavonoids, amino acids, proteins and volatile oils . These secondary metabolites, individually or in combination, would account for the observed pharmacological effects of this plant in this study. In the case of this study, the anxiolytic activity observed with ethanolic extract of *Oxalis corniculata* is possibly due to the presence of flavonoids and tannins in the plant extract. These chemical constituents have been reported to be responsible for anxiolytic effects observed in different plant extracts.

In conclusion, this study shows that ethanolic extract of *Oxalis corniculata* attenuated anxiety parameters in the open-field and plus-maze tests and also inhibited foot shock-induced fighting behavior, thus further supporting the medicinal use of this plant.

Conflict of interest statement

We declare that we have no conflict of interest.

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