Evaluation of acute toxicity, sedative and analgesic effects of *Taverniera glabra* methanolic extract on mice

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Abstract: Present study was conducted on crude methanolic extract of stem and root of *Taverniera glabra*. In Pakistan *T. glabra* is found in the region of Balochistan only. *T. glabra* has numerous therapeutic uses in traditional medicine and it is also used for the pain relief. Current study was carried out to evaluate acute toxicity, analgesic and CNS depressant activity of the plant. Acute toxicity was carried out by oral administration of the *T. glabra* extract from 250 to 2000mg/kg oral dose. Analgesic activity was carried out by acetic acid induced writhing test and formalin test. Central Nervous System (CNS) depressant activity was carried out by exploratory activities (open field activity, cage crossing activity, rearing test) and forced swimming test. Oral administration of the methanolic extract of *T. glabra* was nontoxic at the dose of 1500mg/kg in the acute toxicity test. Exploratory behavior of mice treated with the methanolic extract of *T. glabra* showed sedative effects ($P<0.05$) in open field, cage crossing, traction and rearing test, particularly at the dose of 500mg as compared with standard drug Diazepam. In forced swimming test, mobility time was significantly ($P<0.05$) increased at 500mg/kg oral dose, and results were significant as compared with control. Methanolic extract of *T. glabra* produced significant ($P<0.05$) analgesic effects at the dose of 500mg/kg in the acetic acid induced writhing test and the formalin test. In conclusion, results show that the crude methanolic extract of *T. glabra* possess sedative as well as potent analgesic effects. Present pharmacological studies are the first ever studies conducted on the methanolic extract of *T. glabra.*

Keywords: Analgesic, Sedative, *Taverniera glabra*, Toxicity,

INTRODUCTION

Since ancient times, humans have relied on herbs and plant derived products to live a healthy life, and to cure different diseases. The practice with locally existing herbs for the therapy of numerous ailments is amongst the different inherent traditions around the world. The information of use various parts of herb for a specific purpose is conveyed from one generation to other and relics in the reminiscence of tradition (Ahmad *et al.*, 1999). In Pakistan approximately 6000 plant species with medicinal property are present. Balochistan is the largest province of Pakistan with a total area of 43.6 percent. Balochistan is the indigenous home of many medicinal plants. *Taverniera glabra* Bois is a local plant of Balochistan and roots and stems are used by the people for the cure of different aliments. The *Taverniera* genus belongs to the family of fabaceae and consist of twelve species (Mangalorkar *et al.*, 2013). In Pakistan 4 species of *Taverniera* are present. Locally *T. glabra* is called as Nathi and Lanti (Khan *et al.*, 2005). *Taverniera* species have various medicinal properties such as *T. cunifolia* which is utilized for blood purification, expectorant, antiulcer, wound healing, anti-inflammatory and for spleen tumors (*Mangalorkar et al.*, 2015). At present, no data is available on pharmacological studies of *T. glabra*. In this regard current study was carried out to investigate the acute toxicity, sedative and analgesic effects of the *T. glabra* and the current study is the first report on pharmacological activities of *T. glabra*.

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MATERIAL AND METHODS

Plant material

Plant material was obtained from Turbat city of Balochistan during the month of June. Dr. Mansoor Ahmad, Meritorious Professor, Research Institute of Pharmaceutical Sciences, University of Karachi, identified the plant and Voucher specimen No. M-195 was deposited.

Extraction procedure

The stem and root were dried at 25^{o}C for 15 days, were kept into shade and cut into pieces. The chopped plant parts were saturated in methanol at room temperature for a period of fifteen days. A dark brown semisolid methanolic crude extract was obtained from this solution when it was subjected to filtration and evaporation under reduced pressure by using rotary evaporator.

Animals

This study was conducted using mice, type Swiss albino of both sexes, weighing 25-28grams and which were acquired and kept in Animal House of Centre for Advanced Studies for Vaccinology and Biotechnology, University of Balochistan, Quetta. Standard conditions of

housing i.e. 22±1°C temperature and 12/12 hour light/dark cycle were maintained. They were provide open access to water and were fed on normal pellet diet.

Preliminary phytochemical tests

Test for alkaloids

2g of methanolic extract was dissolved in 5ml of HCl (2N) and residue was filtered. Small amount of Wagner and Mayer's reagent was added, formation of precipitates confirm the presence of alkaloids (Zohra *et al.,* 2012).

Test for saponins

Twenty (20) ml of distilled water and stock solution (1mL) of methanolic extract was added in a test tube and shaken for 15 minutes. Formation of foam layer on top of the test tube indicates the presence of saponins (Zohra *et al.,* 2012).

Test for flavonoids

Formation of blackish red color with treatment of ferric chloride solution (few drops) with test solution, indicates the presence of flavonoids (Bhandary *et al.,* 2012).

Test for glycosides

To five (5) ml methanolic extract, 2ml of glacial acetic acid, FeCl3 5% and H2 SO4 one drop were added. Formation of brown ring shows the presence of glycosides (Joseph *et al.,* 2013).

Test for tannins

5 drops of (1%) gelatin solution containing sodium chloride (10%) was added to one ml of methanolic extract. White precipitate formation shows the presence of tannin (Joseph *et al.,* 2013, Jabbar *et al.,* 2016).

Acute toxicity test

Litchfield, (1949) and Debprasad *et al., (*2003) methods were followed for acute toxicity of methanolic extract. In this experiment the mice were grouped in eight groups (n= 6) and 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 mg/kg were administered orally as a single dose. The mice were separately caged and monitored constantly for 24 hours to observe every consequent effects after administering corresponding dose. Animal deaths were recorded in total number and percentage and the animals were kept under surveillance for another 15 days to notice further possible deaths.

Assessment of exploratory activities

Central nervous system related tests i.e Open field, Head dip, Cage crossing and Rearing test were conducted for exploring exploratory behavior in a quiet and peaceful environment while dividing animals in four groups, and administering oral doses of 0.9% saline for control, 250 & 500mg/kg of *T. glabra* methanolic extract, and Diazepam 2 mg/kg as standard drug.

Open field activity

The area of apparatus consisted of 76 X 76cm^2 with 42 cm height of opaque walls and lines were strained on the bottom which divided it into 25 equal squares which was kept in sound proof room provided with daylight. Method described by Turner (1965), Kennett *et al.,* (1985) and Ahmad *et al.,* (2012) were used. Mice (0.9% saline treated) were placed in center square of the apparatus for 30 minutes, and total number of squares crossed (with all four paws) were counted. In the same way plant extract and standard drug treated mice were observed while avoiding any possible errors.

Cage crossing movement

The method for determination of motor activity of the test animal described by Florence *et al.,* (2000) and Owais *et al.,* (2014) was used. In this test methanolic extract, standard drug, and the control (saline 0.9%) treated mice were placed in a four-sided cage for noticing cage crossing activity for 30 minutes.

Rearing test

The rearing test was carried out to determine the exploratory behavior of the test animals. The method described by Sakina *et al.,* (1990), Sanchez *et al.,* (2002), and Ahmad *et al.,* (2014) was used in which mice were put in a glass beaker of thousand ml (wrinkled with a white paper on its lowermost part). The test animals were observed for upward movements in which they raised their fore paws towards the brim of the beaker in a standing position, and all such movements were counted and recorded.

Traction test

Time taken by the test animal to travel iron rod (one meter in length) after administration of methanolic plant extract was determined in this test. The test was performed in accordance with the Sanchez *et al.,* (2002), Debprasad *et al.,* (2003), and Ahmad *et al.,* (2014). Mice were familiarized to walk on iron rod and then sorted out, and the time consumed by the mice to pass over the iron rod was recorded. Any change in duration of time taken by methanolic extract treated test animals versus other groups describes the traction time.

CNS depressant activity

Forced induced Swimming Test

In order to determine the CNS depressant action of crude extract, method of Turner (1965) and Sanchez *et al.,* (2002) were followed. In this test mice were placed in an apparatus having water marked with upper level at normal temperature (25±2°C) for 6 minutes. It was observed that mice at once started moving their paws when they were place in water. Subsequently, events of immobility such as movements required to retain the body floating, throughout the six minutes were also recorded.

Analgesic activity

Writhing test

The acetic acid induced writhing test method described by Koster *et al.,* (1959), Turner (1971), Ahmad *et al.,* (2012)

and Mehjabeen *et al.,* (2014) was used. The test animals were assembled in four groups containing five animals in each group. A dose of 250 and 500mg/Kg of methanolic extract, while Aspirin (as a standard analgesic drug) at the dose of 300mg/kg and in control group saline solution (0.9%) was administered orally 30 minutes before the intraperitoneal injection of acetic acid. For sorting out writhing behavior, number of writhing and stretching were recorded for 30 minutes after intraperitoneal injection of acetic acid solution (0.6%) 10ml/kg (Jahan *et al.,* 2014).

Formalin test

In accordance with the method described by Hunskaar *et al.,* (1986) and Shibata *et al.,* (1989), test animals were distributed in 4 groups having five animals in each. To each mice a subcutaneous dose of 20µl (0.02ml), 1% formalin solution was injected in right hind paw. In same injected paw, numbers and time (seconds) spent on licking and biting was taken as a sense of pain and was recorded. First phase of neurogenic response occurred immediately after injection of formalin which was measured for five minutes. The second phase response was measured in the last 15 minutes (after injecting formalin). Methanolic extract was administered orally (at dose of 250 & 500mg/kg) and Aspirin 300mg/kg (standard drug) and 0.9% saline water (for control group), 30 minutes before introducing formalin. The mice were assessed for licking or biting the targeted paw for 30 minutes.

STATISTICAL ANALYSIS

Data was Statistically analyzed by method described by Alcaraz *et al.*,(1989) while expressing observe values as Mean ± SEM. Dunnett's t-test was done for significance of difference between means and values of $P<0.05$ were considered significant and $P<0.01$ were considered as highly significant.

RESULTS

Phytochemical tests

The results show that in methanolic extract of *T. glabra*, Alkaloids, Saponins, flavonoids and tannins were present (table 2).

Acute toxicity

The *T. glabra* methanolic extract produced 16% mortality at oral dose of 1500mg/kg and 33.33% mortality at oral dose of 1700, and 2000mg/kg when given orally (table 1).

Exploratory activities

In open field test methanolic extract of *T. glabra* showed decrease activity. In control mice, number of open field activities were 241±3.24, in mice treated with methanolic extract of *T. glabra* at 250 and 500mg/kg, number of activities were decreased to 156.8±6.57 and 133.8±6.99 respectively, as compared with control and standard drug Diazepam.

In cage crossing test, methanolic extract of *T. glabra* showed decrease activity. At 500mg/kg dose number of cage crossing activities were 24.8±3.11 which is comparable with Diazepam (standard drug) 2 mg/kg that produced 21±1.7 numbers of cage crossings.

In traction test, time taken by mice to cross iron rod for control (0.5ml Saline treated mice) was 10.50±1.14, at 250 and 500mg/kg values were 12.4±0.51, 23.2±3.78 respectively, for diazepam (standard drug) values were 40.2±0.73. In rearing activity, number of rearing activities for *T. glabra* methanolic extract reduced particularly 500mg/kg dose i.e. 13.2±2.58, as compared to the control group i.e. 21±1.14 (table 3). Results of exploratory activities show that methanolic extract of *T. glabra* produced sedative effect.

Forced induced swimming test

In this test results show that in control group mobility time was 3.21±0.003 minutes and 2.39±0.003 minutes was immobility time. For methanolic extract 250mg/kg treated group 4.10±0.004 minutes was mobility time and 1.50±0.005 minutes was immobility time, for methanolic extract 500 mg/kg treated group 4.41±0.001 minutes was mobility time and 1.19±0.001 minutes was immobility time. For standard drug diazepam treated group mobility time was 1.32±0.004 and immobility time was 4.28± 0.004. *T. glabra* at the dose 500 mg/kg showed significant ($p<0.05$) results, as compared with control (table 4). Result of forced swimming test shows that the methanolic extract of *T. glabra* have anti-depressant effect.

Analgesic activity

Acetic acid induced writhing test

In writhing test *T. glabra* methanolic extract presented remarkable dose depended inhibition of number of writhes. In control i.e. saline treated animal the mean number of writhes were 79.±3.26 and the same reduced to 51.23±2.74 and 32.7±1.16 in mice introduced oral doses of 250 & 500mg/kg methanolic extract of *T. glabra* correspondingly. Being significant, these results may be compared with aspirin, standard drug, produced 26.5±1.17 writhes (table 5).

Formalin test

In conducting formalin test, significant results were produced by the methanolic extract of *T. glabra*. In first phase mice treated with saline (control group) number of licking and biting were 45.2±1.68 and time spent was 54.8±1.24 seconds and in the second phase 56.8±0.7 counts of lickings and biting with time spent equal to 141.2±5.9 seconds. *T. glabra* at the dose of 250 mg/kg in the first phase 33.4 ±2.36 counts of licking and biting and time spent during this equals 43±2.62 seconds, whereas in

Table 1: Acute toxicity of crude extract of *T. glabra* in mice

Treatment	Oral Dose mg/kg	No. of mice (n)	Percent mortality
Control	0.5ml Saline	6	0%
	250 mg/kg	6	0%
	500mg/kg	6	0%
	750mg/kg	6	0%
Crude extract of T. glabra	1000mg/kg	6	0%
	1250mg/kg	6	0%
	1500mg/kg	6	16%
	1750mg/kg	6	33.33%
	2000mg/kg	6	33.33%

the second phase 41.14±2.0 counts of licking and biting and to increase and intensify the GABAergic inhibition in brain time spent during this equals to 101.10±1.63 seconds. However at increasing the dose as much as 500mg/kg, in the first phase 30±3.98 counts of licking and biting and time spent was equal to 36±2.25 seconds and in the second phase 31.21±0.77 counts of licking and biting and time spent was equal to 61.7±2.86 seconds. While assessing Aspirin (standard drug) in a dose of 300mg/kg, in the first phase 20.8±0.97 licking and biting counts and time spent equal to 18.4±1.03 seconds and in the second phase 21.5±1.61 counts of number of licking and biting and time spent equals to 29.19±0.138 seconds. Methanolic extract of *T. glabra* at the dose of 500 mg/kg presented significant ($P<0.05$) decrease in licking & biting counts and time spent in both phases (table 6).

Table 2: Preliminary Phytochemical tests

S No.	Test	Result
01	Alkaloids	+
02	Saponins	+
04	Flavonoids	+
05	Tannins	+
06	Glycosides	-

+ =Present, **-** = absent

DISCUSSION

Many drugs have failed to show minimum toxicity despite wholehearted efforts to develop new psychiatric drugs for the treatment of anxiety and depression. Taking this fact into consideration, drug products derived from medicinal plants may be an alternative, and can contribute towards the treatment of anxiety, depression and many other psychiatric disorders (Khan *et al.*, 2014).

Toxicity may be defined as an adverse interaction between toxicants and living cells (Antonelli *et al.*, 2015). In acute toxicity test results showed no obvious change at the dose of 1250 mg/kg. Therefor the *T. glabra* extract may be safe at oral dose of 625 mg/kg.

In current study there was significant decrease in the number of open field, rearing and cage crossing activities. Result of our studies suggest that the methanolic extract of *T. glabra* produced sedative effects. *T. glabra* extract may function by inducing hyperpolarization which decreases the rate of fire in the neurons of the brain. It has been hypothesized that it may be due to GABA receptor activation. In phytochemical tests flavonoids were positive. Phytochemical investigation of the plants showed that flavonoids act especially on neurons of the brain are substrates for GABA receptors, and this fact indicates that they act as benzodiazepines, depressing the CNS activity. Thus chemical constituents i.e. flavonoids, Tannins and Alkaloids shows CNS depressant activity (Ripa *et al.*, 2014).

Plant extract of *T. glabra* decreased locomotor activity which is an indicator of CNS depression, because CNS activity is measured by the amount of locomotor activity. As a standard anxiolytic drug, Diazepam is widely used. It is also used as a reference compound for evaluation of behavior and behavior related traits (Khan *et al.*, 2014). Forced Swimming Test (FST) was used as a screening tool due to its flawless reliability and predictive accuracy (Petit *et al.*, 2005). Decrease in immobility time shows the antidepressant like activity of drug (Gupta *et al.*, 2010, Alamgeer *et al.*, 2012). FST showed that methanolic extract of *T. glabra* has antidepressant effect.

Previous studies have showed that mobility and other tests were very sensitive to the drugs known as antidepressant drugs, such as tricyclic antidepressant drugs, monoamine oxidase inhibitors etc. It was documented that these drugs decrease the immobility duration remarkably in the mice when the mice are allowed for swimming in FST or suspended by their tail in TST (Porsolt *et al.*, 1977). It was also found that the extract of *T. glabra* may interact with the dopaminergic or adrenergic system thus acts to reduce the time of mobility. Furthermore, it was observed the methanolic extract of *T. glabra* may causes weakening of stress which is caused by oxidation which is responsible for CNS depression (Alamgeer *et al.*, 2012).

Experimental data obtained in our studies indicates that the methanolic extract of *T. glabra* caused decrease counts of writhes in the writhing test induced by acetic acid, moreover all results were significant ($P<0.05$) at the dose of 500 mg/kg *Marvi et al* The writhing test induced by

Table 3: Effect of methanolic extract of *T. glabra* on exploratory behavior.

Dose mg /kg (body weight)	Open field test	Cage crossing test	Traction test	Rearing test
Control 0.5ml Saline	241±3.24	36.6±2.09	10.50±1.14	21 ± 1.14
Diazepam 2 mg/kg	52.6±2.98**	21±1.7**	40.2±0.73**	7.4±0.68**
T. glabra methanolic extract 250mg/kg	156.8±6.57*	23.2±2.6*	12.4±0.51*	16 ±1.3*
T. glabra methanolic extract 500 mg/kg	133.8±6.99*	24.8±3.11*	23.2±3.78*	13.2± 2.58*

Values are mean number of head dips, cage crossing and rearing in 30 minutes. All values are mean ± SEM; n=5; * = Significant results (P<0.05), ** = highly significant results (P<0.01)

Table 4: Effect of methanolic extract of *T. glabra* on Forced swimming test in mice

Treatment	Dose mg/kg orally	Mobility time Mean No. of observations ±SEM	Immobility time Mean No. of observations ±SEM
Control	0.5ml Saline	3.21± 0.003	2.39 ± 0.003
Crude extract of	250 mg/kg	4.10± 0.004*	1.50 ±0.005*
T.glabra	500mg/kg	4.41 ± 0.001*	1.19±0.001*
Diazepam	2mg/kg	1.32 ± 0.004**	4.28± 0.004**

All values are mean ± SEM; n=5; * = Significant results (P<0.05), ** = highly significant results (P<0.01).

Table 5: Effect of crude extract of *T. glabra* on acetic acid induced writhing in mice.

Treatment	Oral Dose mg/kg	No. Writhes (Mean) ± S.E.M	Inhibition %
Control	0.5ml Saline	79.3±3.26	00
Crude extract of *T. glabra*	250 mg/kg	51.23±2.74*	35.39*
	500 mg/kg	32.7±1.16*	58.76*
Aspirin	300 mg/kg	26.5±1.17**	66.58

Table 6: Effect of crude extract of *T. glabra* inflammatory pain in induced by Formalin in mice

Treatment	Dose mg/kg orally	First Phase Mean No. of observations ± S.E.M		Second Phase Mean No. of observations ± S.E.M	
		No. of Licking & Biting	Time Spent (Seconds)	No. of Licking & Biting	Time Spent (Seconds)
Control	0.5ml Saline	45.2±1.68	54.8±1.24	56.8±0.7	141.2±5.9
Crude extract	250 mg/kg	33.4 ±2.36*	43±2.62*	41.14±2.0*	101.10±1.63*
of *T. glabra*	500 mg/kg	30±3.98*	36±2.25*	31.21±0.77*	61.7±2.86*
Aspirin	300 mg/kg	20.8±0.97**	18.4±1.03**	21.5±1.61**	29.19±0.138**

All values are mean ± SEM; n=5; * = Significant results (P<0.05), ** = highly significant results (P<0.01).

acetic acid is more sensitive and can produce various grades of noxious stimuli in tissue damage (Usha *et al.*, 2013, Al-Harrasi *et al.*, 2014). According to the literature, writhing induced by acetic acid identifies centrally and peripherally acting analgesic compounds (Sekhar *et al.*, 2014). Hence, it can be concluded that *T. glabra* at the dose of 250 and 500mg/kg has both peripheral and central mechanisms of antinociception.

The formalin test is a more accurate model of nociception which investigates the antinociceptive mechanism of the analgesic drugs. The biphasic behavior seems to be associated with two distinct mechanisms. The first phase is the early neurogenic pain phase which is the result of direct stimulation by formalin. It starts immediately after the injection of formalin solution and lasts for 5-10 min. The second phase is the inflammatory phase which is believed to have arose from the spinal neuron hyperactivity involving various mediators (Li *et al.*, 2013, Azadmehr *et al.*, 2013 Al-Harrasi *et al.*, 2014). The crude extract of *T. glabra*, in a dose of 500mg/kg, caused significant (P<0.05) reduction in biting and licking count and the time spent on it in the first and second phase of inflammatory pain induced by formalin. This inhibition in both late and early phases of formalin induced pain indicated the contribution of both mechanisms in the overall analgesic effect, as the pain relieving activity of the some plant extract is exerted due to certain chemical constituents (Alamgeer *et al.*, 2012). To the best of our knowledge there was no data available for pharmacological studies of the plant. Hence current work i.e. the acute toxicity, central nervous system depression and pain relieving activities of *T. glabra* first report in the history of research.

CONCLUSION

Results of current study suggest that methanolic extract of *T. glabra* has potential to be an alternative analgesic agent and having sedative and CNS depressant effect that can be explored for therapeutic improvement as an alternative treatment in conditions associated with psychotic disorders. However extensive studies are required to isolate the chemical constituents responsible for pharmacological effects.

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