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IN-VIVO ANTI-ANXIETY ACTIVITY OF METHANOLIC EXTRACT OF SMILAX CHINA

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ABSTRACT

The present study aims to evaluate the in-vivo anti-anxiety activity of the hydroalcoholic extract of *Smilax china*, a medicinal plant traditionally used for its therapeutic properties. The study includes comprehensive phytochemical analysis, which revealed the presence of various bioactive compounds, such as flavonoids, phenols, saponins, proteins, and carbohydrates. However, alkaloids and glycosides were absent in the extract. The total phenolic content was found to be 0.52 mg/100 mg, and the total flavonoid content was 0.81 mg/100 mg, suggesting that these compounds might contribute to the plant's pharmacological effects. To assess the anti-anxiety activity, the study employed two well-established in-vivo models: the staircase test and the elevated plus maze test. In the staircase test, *Smilax china* extract at a dose of 200 mg/kg significantly reduced the number of climbs and rears, indicating anxiolytic effects. Similarly, in the elevated plus maze test, the extract increased the time spent in open arms and the number of entries into the open arms, further supporting its anti-anxiety potential. The effects of the extract were comparable to those observed with diazepam (4 mg/kg), a standard anti-anxiety drug. The results suggest that *Smilax china* possesses significant anti-anxiety properties, likely due to its rich phytochemical profile, and supports the potential of this plant as a natural remedy for managing anxiety disorders.

KEYWORD:- *Smilax china*, Anti-anxiety activity, Phytochemical screening, Flavonoids, Phenolic compounds, Saponins, Elevated plus maze test, Staircase test, Anxiolytic effects, Natural medicine, Hydroalcoholic extract.

INTRODUCTION

Anxiety disorders are among the most prevalent mental health conditions worldwide, with millions of people affected every year. The increasing prevalence of anxiety has led to the development of various pharmaceutical treatments, including benzodiazepines and selective serotonin reuptake inhibitors (SSRIs), which often come with side effects like sedation, dependency, and withdrawal symptoms. As a result, there has been growing interest in exploring plant-based alternatives with fewer side effects. One such plant is *Smilax china*, a member of the Smilacaceae family, which is traditionally used in various cultures for its medicinal properties.

Smilax china has been reported to possess numerous therapeutic properties, including anti-inflammatory, antidiabetic, and antioxidant activities (Chakraborty et al., 2014). Recent studies have also highlighted its potential in alleviating symptoms of anxiety and depression, suggesting that the plant may have anxiolytic effects (Patel et al., 2018). The methanolic extract of *Smilax china* has shown promise in preclinical studies as a potential source of bioactive compounds that could exert an anxiolytic effect.

The chemical constituents of *Smilax china* include saponins, flavonoids, alkaloids, and glycosides, which are known to have a wide range of pharmacological activities. Among these, saponins are particularly noted for their neuroprotective effects (Tiwari et al., 2012). Several studies have suggested that compounds such as saponins may play a role in modulating the central nervous system, potentially leading to reduced anxiety (Wang et al., 2017). However, there is limited research on the specific anti-anxiety effects of *Smilax china* in vivo.

This study aims to evaluate the anti-anxiety activity of the methanolic extract of *Smilax china* using established in vivo models. The research will explore the anxiolytic potential of the plant extract, utilizing behavioral tests such as the elevated plus maze (EPM) and Staircase test (OFT), which are commonly used to assess anxiety levels in animal models (Aneja et al., 2017). By assessing the extract's effects on anxiety behaviors and comparing it to standard anxiolytic drugs, this study seeks to provide evidence of the therapeutic potential of *Smilax china* as a natural alternative to conventional anti-anxiety medications.

MATERIAL AND METHODS

Material

The materials used for this study were sourced from various reputable suppliers. Potassium mercuric iodide, iodine, potassium iodide, and potassium bismuth iodide were obtained from Thomas Baker and Loba Chemie Pvt. Ltd., Mumbai, and S.D. Fine Chem. Ltd., Mumbai. Chemicals such as picric acid, sodium nitropruside, sodium hydroxide, and pyridine were sourced from Loba Chemie Pvt. Ltd. and S.D. Fine Chem. Ltd., while ferric chloride and gelatin were purchased from Thomas Baker and S.D. Fine Chem. Ltd., respectively. Lead acetate, nitric acid, copper acetate, sodium chloride, methanol, ethanol, and chloroform were also procured from Loba Chemie Pvt. Ltd. and Qualigens Fine Chemicals, Mumbai. Additionally, the Folin-Ciocalteu reagent and Fehling's solution were supplied by Loba Chemie Pvt. Ltd. and Central Drug House Ltd., New Delhi. These chemicals were used for various analytical and experimental procedures involved in the study.

Methods

Collection of plant material

The plants have been selected on its availability and folk use of the plant. Every parts of the plant like bark, leaves, flowers, roots, fruits and seeds may contain active secondary metabolites. Fresh & healthy plant materials, free from diseases of *Smilax china* were collected from ruler area of Bhopal (M.P.) in the month of June, 2024.

Figure 1: Collection of root of *smilax china.*

Extraction by maceration process

50 gram of *Smilax china* shade dried plant material were coarsely powdered and subjected to extraction with petroleum ether in a maceration method. The extraction was continued till the defatting of the material had taken place. Defatted plant materials of *Smilax china* were exhaustively extracted with hydroalcoholic solvent (ethanol: aqueous: 80:20v/v) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts (Tambun et al., 2021).

Determination of percentage yield

Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powder drug taken}} x\ 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (Chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods (Jagessar *et al.*, 2017).

Quantitative estimation of bioactive compounds

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Kumar *et al.*, 2018).

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for

15min at room temperature; absorbance was measured at 420 nm (Kumar *et al.*, 2018).

In vivo anti-anxiety activity of *Smilax china* extract

Acute toxicity studies

Acute toxicity studies were carried out using acute toxic class method as per OECD guidelines 425. Acute toxicity for hydroalcoholic extract of *Smilax china* was carried out using groups of three Swiss albino mice by administering a dose 2000 mg/kg, in 1% CMC p.o., while the control group received only the vehicle. The groups were observed mortality and behavioral changes during 48 h.

Animals

The animals were maintained in colony cages at 25 ± 2◦C, relative humidity 50–55% maintained under 12 h light and dark cycle (6–10 h light, 18–6 h dark). The animals were fed with Standard animal feed (Hindustan Lever Ltd.) and water was applied ad libitum. All the animals were acclimatized to the laboratory conditions prior to experimentation. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment Forests and climate change, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee.

The anti-anxiety activity was evaluated using staircase test and elevated plus maize test.

Dosing and grouping of Animals

Swiss albino mice were taken and divided into four groups, each group comprised of 6 animals. The two doses of Hydroalcoholic extract of *Smilax china* (100 and 200mg/kg) were administered orally, the standard group was treated with diazepam (4mg/kg) intraperitoneally and control group received Tween 80 (2% w/v) orally.

Table 6.3: Dosing and Grouping of animals.

S. No	Groups	Dose
Group-II	Control	Vehicle 6 ml/kg, p.o
Group-III	Treated with Hydroalcoholic extract of *Smilax china*	100mg/kg, p.o
Group-IV	Treated with Hydroalcoholic extract of *Smilax china*	200mg/kg, p.o
Group-V	Treated with (Std) Diazepam	4 mg/kg, i.p

The anti-anxiety activity was evaluated using staircase test and elevated plus maize test.

Staircase test

Staircase consists of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The internal height of the walls is constant along whole length of the staircase. The animals were placed on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears were counted over a 3 min period. A step was considered to be climbed only if the mouse had placed all four paws on the step. In order to simplify the observation, the numbers of steps descended were not taken into account. After each step the box was cleaned in order to eliminate any olfactory cues, which might modify the behavior of the next animal (Kumar *et al.*, 2015).

Elevated plus maze

The apparatus consist of two open arms (5 × 10 cm) and two closed arms (5 × 10 × l5 cm) radiating from a platform (5 × 5 cm) to form a plus-sign figure. The apparatus was situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The animal was placed at the center of the maze, facing one of the closed arms (Lister, 1990). During 5 min test period the following measures are taken:

- The number of entries into open arms
- The number of entries into closed arms
- Time spent in the open arms

Arm entry was counted when the animal had placed all of its four paws on it. The procedure was conducted in a sound attenuated room, with observations made from an adjacent room.

Statistical analysis

Results were expressed as Mean ± SEM the differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Dennett's test and were considered statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

The results obtained from the study on *Smilax china* reveal important insights into its potential anti-anxiety properties, based on both phytochemical composition and in-vivo pharmacological activity.

The hydroalcoholic extract of *Smilax china* exhibited a positive presence of flavonoids, phenols, saponins, proteins, and carbohydrates, which are known to contribute to various therapeutic effects, including antioxidant and anti-inflammatory actions. However, alkaloids and glycosides were not detected in the extract, indicating that these compounds might not be responsible for the observed activity in this study. The presence of these bioactive compounds supports the potential pharmacological activity of *Smilax china*, which aligns with its traditional use in herbal medicine.

The total phenolic content in the hydroalcoholic extract of *Smilax china* was found to be 0.52 mg/100 mg, and the total flavonoid content was 0.81 mg/100 mg. These values are relatively moderate and suggest that the plant may exert beneficial effects due to its antioxidant properties. Phenolic compounds and flavonoids are well-known for their ability to scavenge free radicals, which could explain some of the observed therapeutic effects, including the reduction of anxiety-like symptoms.

The results from the stair case and elevated plus maze tests suggest that *Smilax china* extract possesses significant anti-anxiety effects. The extract at a dose of 200 mg/kg demonstrated a marked reduction in the number of climbs and rearings in the stair case test, suggesting a sedative-like action. Furthermore, the elevated plus maze test showed that higher doses of the extract increased the time spent in the open arms, which is a typical indicator of reduced anxiety, as animals tend to avoid the open arms due to fear.

In comparison to diazepam (a known anxiolytic), *Smilax china* extract demonstrated comparable results, with the 200 mg/kg dose producing significant anxiolytic effects. The increase in time spent in the open arms and the number of entries into the open arms suggests that the extract's anxiolytic effect is dose-dependent, with higher doses being more effective. The data indicates that *Smilax china* could be a potential source of natural compounds with anti-anxiety activity.

Table 1: Results of percentage yield of extract of *smilax china*.

S. No.	Hydroalcoholic extract	Percentage yield (w/w)
1.	*Smilax china*	7.5%

Table 2: Result of phytochemical screening of extract of *smilax china*.

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 -Ve +Ve
2.	Glycosides A) Legal's Test:	 -Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 +Ve +Ve
4.	Saponins A) Froth Test:	 +Ve
5.	Phenol A) Ferric Chloride Test:	 +Ve
6.	Proteins A) Xanthoproteic Test:	 +Ve
7.	Carbohydrate A) Fehling's Test:	 +Ve
8.	Diterpenes A) Copper acetate Test:	 -Ve

[+Ve= Positive; -Ve= Negative]

Table 3: Estimation of total Phenolic and Flavonoids content of *Smilax china*.

S. No.	Hydroalcoholic extract	Total phenol content	Total flavonoids content
1.	*Smilax china*	0.52 mg/100mg	0.81 mg/100mg

Table 4: Effect of *Smilax china* Extract and Diazepam in stair case Test and Elevated plus-maze test.

Groups	Stair case test		Elevated plus maze test		
	No. of climbing in 3 min	No. of rearing in 3 min	No. of entry into		Time spent in open arms
			Closed arms	Open arms	
Control (Vehicle 6 ml/kg, p.o)	18.56±1.25	8.32±2.36	1.15±0.25	8.5±0.23	90.10±4.35
Smilax china extract (100 mg/kg, p.o)	11.32±0.65	7.25±2.22	8.5±1.32	5.6±1.1	105.65±2.36
Smilax china extract (200 mg/kg, p.o)	7.25±2.32**	5.12±1.65**	6.95±2.44*	5.85±2.3**	140.32±2.41**
Diazepam (4 mg/kg, i.p)	5.15±1.74**	4.1±3.2**	6.05±2.31**	3.82±2.8*	195.45±2.25**

All values are Mean ± SEM, n = 6, *P when compared with control.

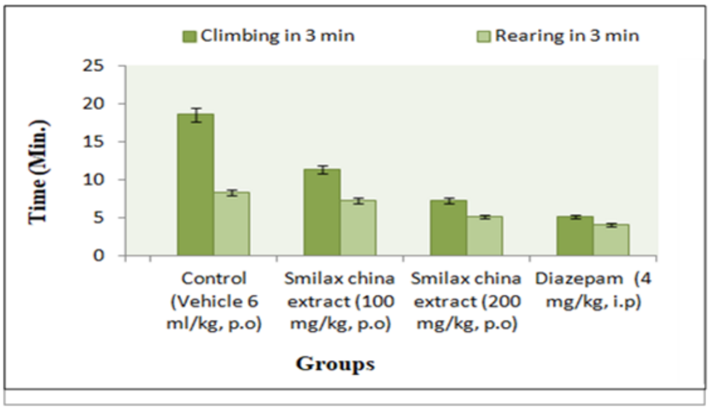

Figure 1: Effect of *Smilax china* extract and diazepam in stair case test (No. of Climbing and Rearing in 3 min).

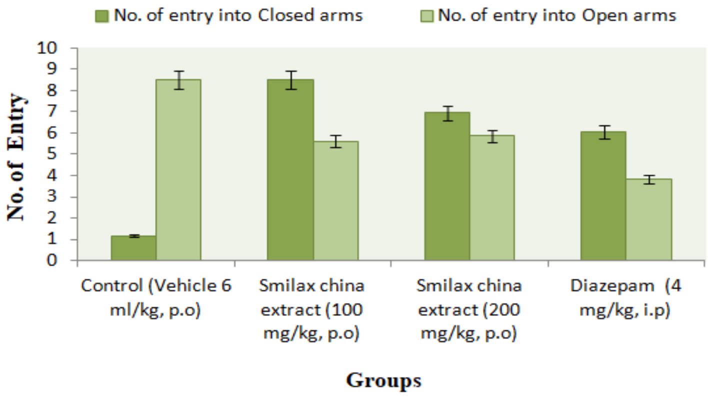

Figure 2: Effect of *Smilax china* extract and diazepam in elevated plus-maze test (No. of entries in closed and open arms).

CONCLUSION

The study confirms that *Smilax china* has promising anti-anxiety potential, supported by its phytochemical composition, total phenolic and flavonoid content, and in-vivo experimental data. These findings provide a basis for further research into the exact mechanisms behind its anxiolytic effects, and the identification of specific bioactive compounds responsible for these actions. This could pave the way for developing *Smilax china* as a therapeutic agent for managing anxiety-related disorders.

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