

EVALUATION OF ANXIOLYTIC ACTIVITY OF METHANOLIC EXTRACT OF *CANNABIS SATIVA*

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ABSTRACT

Anxiolytic Activity of Methanolic Extract of *Cannabis Sativa Linn.* leaves were evaluated in wistar rats. The experimental model used was Elevated plus maze Apparatus and Elevated Zero maze Apparatus.¹ Diazepam (2 mg/kg., I.P.) was used as Standard. All the three extracts (5mg/kg, P.O., 10 mg/kg. P.O. and 15 mg/kg P.O) were given orally and found to have significant (P<0.01) anxiety activity.

The *cannabis Sativa Linn.* Leaves extract was found to have improves the anxiety related behavior in rat.

Key words – *Cannabis Sativa Linn.*, Leaves, anxiety.

INTRODUCTION

The Plant *Cannabis Sativa Linn.* belongs to family cannabinaceae. The plant are the rich source of Cannabinoids terpenoids, hydrocarbons, sugars, nitrogenous compounds, Flavonoids, Fatty acids, amino acids, Ketones, esters, aldehydes glycoproteins, enzymes, Steroids, alcohols, Pigments and Vitamin (Vitamin K)^{2,3} The mineral elements of this plant are used for the treatment of nervous disorders, gout, neuralgia, rheumatism, insomnia and Glaucoma,⁴ Recently it show gastric protective activity, psychoactive activity, in alzheimer's disease, analgesic activity, sleep disorder antidepressant activity, anti epileptic activity, and in multiplesclerosis. The present work is an effort to show that the leaves of *cannabis Sativa Linn.* has the anxiolytic activity.

MATERIALS AND METHODS

The leaves of plant were collected from local region of Bareilly (U.P.). The identification and authentication of plant was performed in the Department of Botany, M.J.P. Rohilkhand University, Bareilly (U.P.). Leaves of Mature plants were taken and clean with the help of tap water and after that leaves were dry under shade from 20 to 25 days. The dried leaves were converted in to powder form with the help of mechanical support. The dry leaves powder were extracted with methanol solvent for 1, 2 and 3 hours by using soxhlet apparatus for complete extraction.

The extract was filtered with the help of whatsmann filter paper and excess Solvent evaporate with the help of rotary evaporator.

The 30 Wistar rats (200-225 gm) were weighed and divided into five groups, each group Containing 6 rats. The animals were house in groups of 5 and maintained temperature and light controlled environment on 12:12 h light dark cycle with free access of food and water. All the solutions were freshly prepared and administered daily in animals for five days by oral route.^{5,6}

Group – I – It was treated with 2ml/kg of Normal Saline for 1 to 5 days.

Group – II – It was treated with 2 mg/kg I.P. of Diazepam for 1 to 5 days.

Group – III – It was treated with 5 mg/kg. Oral of Cannabis leaves extracted for 1 to 5 days.

Group – IV – It was treated with 10 mg/kg. Oral of Cannabis leaves extract for 1 to 5 days.

Group – V – It was treated with 15 mg/kg. Oral of cannabis leaves extract for 1 to 5 days.

RESULTS AND DISCUSSION

All results were expressed as mean \pm SEM. Data was analyzed using one way ANOVA.

1-ELEVATED PLUS MAZE APPARATUS

Each values represent mean \pm SEM (n=6), compared to control in elevated plus maze apparatus during the time session of 5 minutes. Test T1, T2 and T3 group consist of six animals, in each group treated with *Cannabis sativa* of dose 5mg/kg, 10mg/kg and 15mg/kg respectively for five days. After treatment they

were tested in elevated plus maze apparatus to evaluate its anti anxiety effects during the time session of 5 minutes. The times spend in open arm and number of entry in open arm by the animal in apparatus is noted down. The result is significant p-1.467 at $p < 0.01$.

Table-1- Elevated plus maze apparatus

Treatment	Time spend in open arm (sec)	No. of entry in open arm
Control	10.48±4.14	4.48±0.61
Standard	56.16±9.21	24.66±2.49
Test-I	18.11±5.22	9.66±2.33
Test-II	34.90±6.30	15.88±2.44
Test-III	38.10±8.22	19.56±2.48

2-ZERO MAZE APPARATUS

Each values represent mean \pm SEM (n=6), compared to control in zero maze apparatus during the time session of 5 minutes. Test T1, T2 and T3 group consist of six animals, in each group treated with *Cannabis sativa* of dose 10mg/kg and 15mg/kg respectively for five days. After treatment they were tested in zero maze apparatus to evaluate its anti anxiety effects during the time session of 5 minutes. The time spends in open arm and number of stretching by the animal in apparatus is noted down. The result is significant p-0.0014 at $p < 0.01$.

Table-2- Zero Maze Apparatus

Treatment	Time spend in open arm (sec)	No. of Streching
Control	34.83±8.48	11.66 \pm 2.61
Standard	88.83±6.02	4.66±2.49
Test-I	32.16±10.79	10.66±2.33
Test-II	78.80±8.26	8.66±2.42
Test-III	92.02±8.26	6.33±2.33

DISCUSSION

The two experimental models of anxiety, elevated plus maze and elevated zero maze are based on the assumption that unfamiliar, non-protective and brightly lit environmental stress provokes inhibition of normal behavior. This normal behavioral inhibition is further augmented in the presence of fear or anxiety like state.

In the elevated plus maze, the open arms are more fear provoking than the closed arms. The ratio of entries, time spent and rearing behavior in open arms to closed arms reflects the safety of closed arms with relative fearfulness of open arms. The reduction in entry, time spent, rearing in open arms, ratio of open arm to total arm entries and increased defecation are the indications of high level of fear or anxiety. Anxiolytic drugs increase the proportion of entries, time spent and rearing in open arms. They also increase the ratio of open arm to total arm entries.

The test compound, *Cannabis Sativa* (5mg, 10mg, 15mg/kg) increased the number of entries and time spent in open arms in the elevated plus maze paradigm. In elevated plus maze apparatus the test drug significantly time spend in open arm. These behavioral changes produced by the test compound Cannabis Sativa were comparable to those produced by diazepam.

In the elevated zero maze the ratio of entries, time spent and stretching behavior in open arms to closed arms reflects the safety of closed arms with relative fearfulness of open arms. The reduction in time spent, stretching, and increased defecation are the indications of high level of fear or anxiety Anxiolytic drugs increase the proportion of, time spent and stretching. They also increase the ratio of open arm to total arm entries.

On behalf of this study it can determine methanolic dose of *Cannabis Sativa* 5mg, 10mg, 15 mg/kg to have anti-anxiety activity in rats. This activity might be due to enhanced level of serotonin and norepinephrine neurotransmitter in the brain of rats. This study is supported by earlier studies of *Cannabis Sativa* on anxiety. *Cannabis Sativa* leaves extract contain cannabinoids in which act on anxiety by decreases Corticotropin releasing factor (CRF) expression in the brain. As CRF is commonly implicated in the high anxiety.

CONCLUSION

The present study was designed to evaluate the effect of *Cannabis sativa* extract. Anxiety related behavior in rat was evaluated by using elevated plus maze test and elevated zero maze test and elevated zero maze test activity. All the test solutions was freshly prepared daily and administered in rats by intraperitoneal route and oral routes.

Normal saline administered group showed no significant difference in behavior of rats

Diazepam (2mg/kg) administered groups shows a significant increase in open arm and decreases the number of entry and number of time spent in close arm as compared to normal saline administered group and *Cannabis sativa* extract administered group. This result shows that diazepam improves the anxiety related behavior in rat.

Cannabis sativa extract (5mg/kg), *Cannabis sativa* extract (10mg/kg) and *Cannabis sativa* extract (15mg/kg) administered group were found to be significantly increases the number of time spent and number of entry in open arm as compared to normal saline (2ml/kg) administered group. While it decreases the number of entry and time spent as compared to normal saline (2ml/kg) administered group. This result stated that *Cannabis sativa* extract improves the anxiety related behavior in rat. Finally it may be concluded that *Cannabis sativa* extract a protective effect and may attribute to its anti-anxiety effect.

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