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# A Study of Central Nervous System (CNS) Effect of *Xanthium Strumarium* in Swiss Albino Mice

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## ABSTRACT

***Xanthium strumarium* L.** (Family: Asteraceae), a common weed in North America, Brazil, China, Malaysia, and the drier regions of India, is a medicinal plant. The herb has historically been used to treat a variety of illnesses. Traditional medicine has used extracts of the entire plant, particularly the leaves, roots, fruits, and seeds, to treat conditions like leucoderma, epilepsy, salivation, chronic malaria, rheumatism, tuberculosis, allergic rhinitis, sinitis, urticaria, rheumatoid arthritis, constipation, diarrhoea, leprosy, lumbago, pruritis, bacterial infections, and fungal infections. In this present study, we investigated neurobehavioral activity of ethanol extract of *Xanthium strumarium* L in mice as a part of a psychopharmacological screening of this plant. The effects of the plant extract on CNS were studied by using Elevated plus maze test, Hole board test, Hole cross test, open field test. The overall results suggest that the ethanol extract of *Xanthium strumarium* have significant effect on CNS. However, future efforts should concentrate more on in vitro and in vivo studies in order to confirm traditional insight in the light of a rational phytotherapy.

***KEYWORDS***: *Xanthium strumarium, statistical analysis, EPM test, effect, CNS*

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## 1. INTRODUCTION

Since the start of time, nature has been the most reliable source of possible pharmacological ingredients. The enormous structural variety of molecules derived from natural sources is used as a "lead" source for the creation of novel medications that have better pharmacological action and fewer negative effects. Now it is clear that neurological conditions are major global health issues. This is reflected in the Global Burden of Disease Study, jointly published by the World Health Organization and othergroups. Therefore, there is a strong need of new antioxidants, anti- nociceptives, and CNS depressants from natural sources for the development of novel drug products. The word "xanthium" refers to the seedpods, which change from green to yellow as they ripen (later they become deep yellow to brown), and is derived from the ancient Greek words "xanthos," which means yellow, and "strumarium," which means "cushionlike swelling."[1]. Because of the way its fruit, which resembles a cow's toe, is shaped, it is frequently referred to as chotagokhru. Due to its usage in treating the common condition hemicrania, this weed is known as adhasisi in various parts of India. There are 25 species in the genus Xanthium, all of which are native to America. In Europe, North America, and Brazil, Xanthium spinosum Linn. and X. strumarium Linn. are used as medicines; Xanthium canadens Mill. is used in North America and Brazil, and X. strumarium Linn. in China, India, and Malaysia[2]. More than 170 chemical compounds, including sesquiterpene lactones, phenols, glycosides, alkaloids, fatty acids, and others, have been extracted and identified from the plant X. strumarium as a result of the numerous research that have been done thus far on its pharmacology and phytochemistry[3]. Additionally, growing proof suggests that X. strumarium has a broad range of pharmacological effects, including effects on the nervous and digestive systems, as well as analgesic and anti-inflammatory, antioxidant, hypoglycemic, anti-cancer, antibacterial and antifungal, anti-trypanosomal, and anti-tussive activities[4]. The herb itself is thought to be harmful, however washing and cooking get rid of the poisonous

elements[5]. Attractyloside and chlorogenic acid are employed as the quality indicator substances for evaluating the quality of the fruits of X. strumarium, which are still often used in Traditional Chinese Medicine (TCM) and are mentioned in the CHP[6]. In this present study, we investigated neurobehavioral activity of ethanol extract of *Xanthium strumarium* L in mice as a part of a psychopharmacological screening of this plant. The effects of the plant extract on CNS were studied by using Elevated plus maze test, Hole board test, Hole cross test, open field test. The overall results suggest that the ethanol extract of *X. strumarium* have significant effect on CNS. However, future efforts should concentrate more on in vitro and in vivo studies in order to confirm traditional wisdom in the light of a rational phytotherapy.

### 1.1. Scientific Classification

| | |
|---|---|
| Kingdom: | Plantae |
| Division: | Angiosperms |
| Sub-Division: | Eudicots |
| Class: | Asterids |
| Order: | Asterales |
| Family: | Asteraceae |
| Genus: | *Xanthium* |
| Species: | ***X. strumarium*** |

## 2. XANTHIUM STRUMARIUM SIDE EFFECTS

X. strumarium is poisonous to mammals. It is reported to have medium to strong allergenic effects. The toxic principle is a sulphated glycoside, carboxyatractyloside, found in the seeds and during the two-leaf seedling stage. The mature plant is reported as non-toxic, although toxicosis has been reported in cattle which had ingested mature plants with burs despite the general belief that ingestion of burs should be limited by mechanical injury during mastication[7-8]. It is known to result in losses in cattle, horses, goats, pigs, sheep, and swine, as well as reduced weight gain in poultry[8-9]. The plant is a potential cause of sudden death in pigs extensively reared in Zimbabwe, which is revealed by an investigation in which six healthy porkers of the Mukota breed were fed and libitum either crushed burs (fruits) or the two-leaf seedling stage of Xanthium.

## 3. BOTANICAL DESCRIPTION

Warmer climates are where X. strumarium typically thrives. It is an annual herb that can grow up to 1 m tall and has a short, sturdy, hairy stem. The leaves are broadly alternating and triangular-ovate or suborbicular in shape, with irregular lobes and rather inconspicuous teeth. They are 5–15 cm long, frequently three-lobed, have prominent veins, a long petiole, and are scabrous on both sides. Fruits have two hooked beaks and hooked bristles and are obovoid in shape. They are surrounded in a hardened involucre. August through September are India's flowering months. Due to the fruits' hooked bristles and two robust hooked beaks, this plant is easily spread by animals. The seeds ripen from August to October, and it blooms from July to October. Insects fertilise the monoecious flowers, which are. The plant reproduces on its own. When the fruits are ready, they are picked and dried for use[10].


**Figure 1. Xanthium strumarium L. A–D represent the whole plants (A), leaves (B), inflorescence (C) and fruits (D) of X. strumarium L.**

## 4. MATERIALS AND METHODS

### 4.1. Plant material

The plant samples were collected from the fruit of ***Xanthium strumarium*** .

### 4.2. Preparation of the extract

We dried The fruits parts at room temperature under shade for between 9 - 14 days and thencrushed into coarse powder with a pestle and mortal. The powdered fruit was exhaustively extracted with methanol using soxhlet apparatus. The solvent in both cases were removed at reduced pressure.

### 4.3. Animals

We used Swiss albino mice (28-35g) of male sex for the study. The animals were kept and maintained under laboratory conditions of temperature, humidity and light, and were allowedfree access to food and water. All experiments were conducted in accordance with animal use ethics as accepted internationally.

### 4.4. Drug

We used Benzodiazepum tablet(5mg/body weight) as a standard.

### 4.5. Dose and Route of Administration

The mice were administered at a dose o 250 gm/kg body weight of Xanthium strumarium which

considered as 1X dose and another 500 gm/kg body weight of Xanthium strumarium which considered as 2X dose.

The route of administration was per oral (p.o)

**Location and duration:** This study conducted at pharmacology lab of Jahangirnagar University from october 2022 to march 2023.

## 5. EXPERIMENTAL PROCEDURES FOR CNS ACTIVITY TEST

### 5.1. Elevated plus Maze Test:

The elevated plus maze (EPM) is a test that assess**es anxiety in lab animals that** often employs rats as a screening test for potential anxiolytic or anxiogenic substances and as a general research tool in studies of neurobiological anxiety, including PTSD and TBI[11].

**Principle:** The general principle is that the more "anxious" the subject are, the less likely they will be to explore and uncomfortable, risky or threatening environment.

**Procedure:** The elevated plus maze (EPM) is an anxiety test for lab animals that often employs mice as a screening test for potential anxiolytic or anxiogenic substances and as a general research tool in studies of neurobiological anxiety, such as PTSD and TBI. The model is based on the test animal's aversion to open spaces and tendency to be thigmotaxic. In the EPM, this anxiety is expressed by the animal spending more time in the enclosed arms.

### 5.2. Hole Board Test

**Principle:** The basic idea behind the test is that unfamiliar outdoor situations cause animals to exhibit a pattern of behaviour that includes emotional defecation, movement, and exploration (head dipping through the hole during exploration).

**Procedure:** The holeboard test is mainly used for assessing exploratory behaviors in rodents. The animal is placed on an arena with regularly arranged holes on the floor Then, over a brief period of time, the frequency and duration of spontaneously evoked hole-poking behaviour was measured.

### 5.3. Hole cross test

**Principle:** This study examines the effects of the medicinal plant Xanthium strumarium on the central nervous system by measuring locomotor activity. The experiment shows the impact on the animals' locomotor activity.

**Procedure:** Each mouse was immediately placed on one side of the specified instrument, after oral administration of test drugs. The spontaneous movement of the mice from one chamber to other through the hole was observed for 3 **min**. The observation was conducted at 0, 30, 60, 90, and 120 min.

### 5.4. Open field test

**Principle:** The test is set up so that the test animals are not given any pacific tasks to complete. It has been reported that in absence of any specific task to perform, the behavior of a given animal tends to maintain that inner activation level that is at precise times in consistence with theactual level of activation of animals.

**Procedure:** In rodent models of CNS diseases, the Open Field task is a straightforward sensorimotor test used to assess general activity levels, gross locomotor activity, and exploratory preferences. An evaluation is conducted in a box made of square, white Plexiglas. The animal is placed in the arena and allowed to freely move about for 10 minutes while being recorded by an overhead camera. The footage is then analyzed by an automated tracking system for the following parameters: distance moved, velocity, and time spent in pre-defined zones. The Open Field test can be used to phenotype transgenic mouse strains and assess how new chemical substances affect overall activity.

### Statistical Analysis

Data were analyzed by One way ANOVA following Dunnet's post hoc test using SPSS 16.0 and presented as Mean±SEM, (n=6). * ($p<0.05$) = significance, ** ($p<0.01$) = highly significance, and *** ($p<0.001$) = very highly significance as compared to control group.

## 6. RESULT AND DISCUSSION ON CENTRAL NERVOUS SYSTEM PROPERTY

### 6.1. Elevated plus maze test:

The elevated plus maze test is based on mice's innate dislike of elevated and open spaces as well as on their spontaneous natural exploration of new environments.

#### 6.1.1. in open arm:

During 1 hour later for standard dose, the result was highly significant.

And during 2 hours and 3 hours later for standard doses, the result was very highly significant. This indicates, it has better effects on CNS system.

During 2 hours and 3 hours later for 1X and 2X doses, the result was very highly significant. This also indicates, the medicinal plant Xanthium strumarium has the best effects on CNS system.

**Table 1: Effect of XS 1x and XS 2x on the time spent in Open arm in Elevated plus Maze test inmice.**

| Group | Time Spent in Open Arm (Mean±SEM) | | | |
|---|---|---|---|---|
| | -30 min | 1 Hour | 2 Hours | 3 Hours |
| Control | 61.33±4.49 | 52.67±1.87 | 49.33±2.03 | 60.0±1.88 |
| STD (Benzodiazepum 5mg/kg) | 59.33±2.87 | 175.0±45.83** | 13.33±4.90*** | 16.33±4.98*** |
| XS 1x | 57.00±7.57 | 18.67±4.49 | 11.67±2.68*** | 9.50±1.73*** |
| XS 2x | 57.83±3.55 | 28.17±5.08 | 21.67±5.19*** | 30.17±6.37*** |

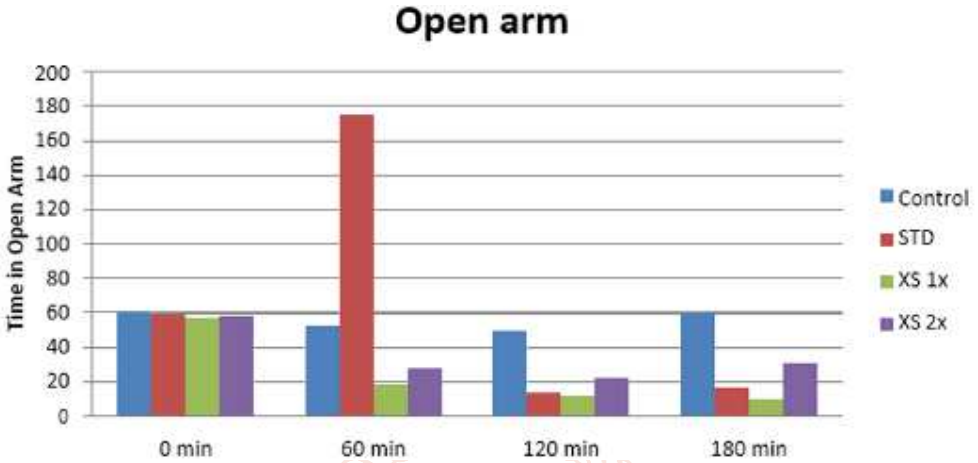


**Figure 2: Effect of XS 1x and XS 2x on the time spent in Open arm in Elevated Plus Maze test in mice.**

### 6.1.2. In closed arm:

During 2 hours and 3 hours later for Standard dose, the result was very highly significant. Thisindicates the best CNS effects of the plant extract.

During 2 hours and 3 hours later for 1X and 2X doses, the result was also very highly significant. This also indicates the best CNS effects of the plant extract.

**Table 2: Effect of XS 1x and XS 2x on the time spent in Close arm in Elevated Plus Maze test inmice**

| Group | Time Spent in Close Arm (Mean±SEM) | | | |
|---|---|---|---|---|
| | -30 min | 1 Hour | 2 Hours | 3 Hours |
| Control | 118.67±4.49 | 127.33±1.87 | 130.67±2.03 | 120.0±1.88 |
| STD (Benzodiazepum 5 mg/kg) | 120.67±2.87 | 160.33±2.75 | 166.67±4.90*** | 162.0±4.32*** |
| XS 1x | 121.33±8.04 | 161.33±4.49 | 168.33±2.68*** | 170.50±1.73*** |
| XS 2x | 122.17±3.55 | 153.50±5.16 | 158.33±5.19*** | 149.83±6.37*** |

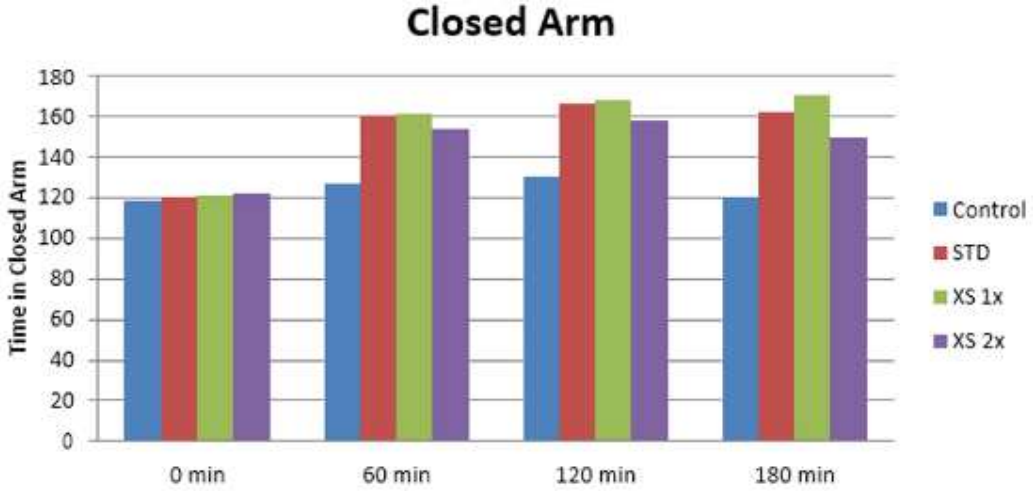


**Figure 3: Effect of XS 1x and XS 2x on the time spent in Close arm in Elevated Plus Maze test in mice.**

### 6.2. Hole board test

This experiment is carried out to get a clear picture of the CNS effect under consideration on the pattern of behaviour by spontaneous ambulatory activity, exploratory activity and emotional defecation of animals. This experiment present with a different and more complex environment to explore.

### 6.2.1. Area Exploration:

There is no significance result for area exploration for total time periods for control, standard, 1X and 2X doses. This indicates there have no effect of the plant extract on CNS system.

**Table 3: Effect of XS 1x and XS 2x on the "Ambulation" in Hole Board test in mice.**

| Group | Number of travel of "Ambulation" (Mean±SEM) | | | | |
|---|---|---|---|---|---|
| | -30 min | 30 min | 60 min | 120 min | 180 min |
| Control | 9.67±1.20 | 11.00±2.67 | 10.67±2.59 | 12.17±4.16 | 10.50±3.73 |
| STD (Benzodiazepum5mg/kg) | 12.00±2.58 | 9.67±3.44 | 5.50±2.77 | 4.5±1.11 | 6.0±1.0 |
| XS 1x | 10.83±3.27 | 10.67±3.91 | 13.50±4.63 | 11.00±3.68 | 10.17±3.22 |
| XS 2x | 8.50±3.19 | 9.33±3.30 | 13.17±3.98 | 12.33±5.93 | 12.33±5.58 |

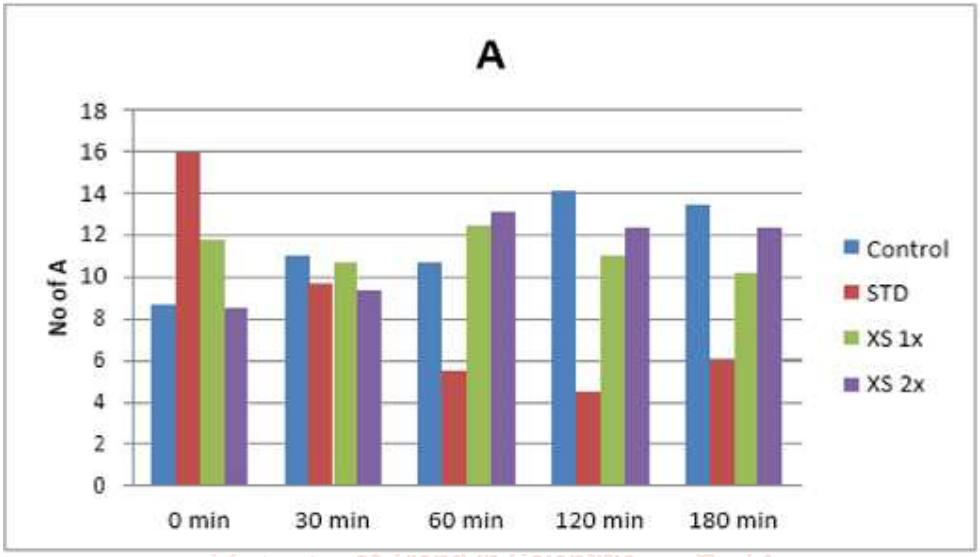


**Figure 4: Effect of XS 1x and XS 2x on the "A" in Hole Board test in mice.**

### 6.2.2. Head Dipping:

There is moderate significant result for Head dipping for total time periods for control, standard,1X and 2X doses.

**Table 4: Effect of XS 1x and XS 2x on the "Head Dipping" in Hole Board test in mice.**

| Group | Number of "Head Dipping" (Mean±SEM) | | | | |
|---|---|---|---|---|---|
| | -30 min | 30 min | 60 min | 120 min | 180 min |
| Control | 11.17±5.15 | 7.33±3.66 | 7.67±2.66 | 8.83±3.42 | 8.33±2.80 |
| STD (Benzodiazepum5 mg/kg) | 9.17±2.21 | 1.33±0.42 | 0.33±0.21 | 0.83±0.31 | 1.33±0.42 |
| XS 1x | 8.33±3.33 | 5.50±2.17 | 7.50±3.06 | 6.83±3.26 | 4.83±2.03 |
| XS 2x | 12.00±4.31 | 5.33±1.89 | 6.50±2.95 | 6.50±3.94 | 5.33±3.03 |

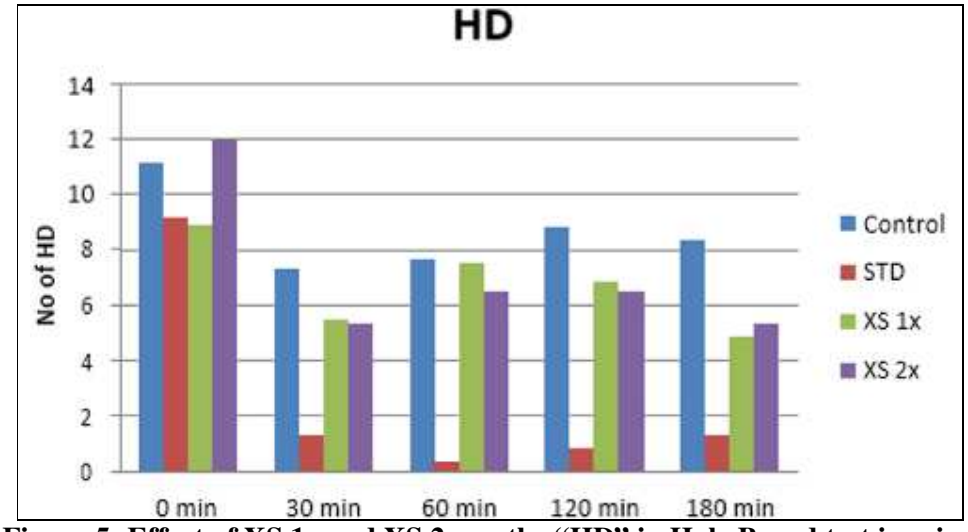


**Figure 5: Effect of XS 1x and XS 2x on the "HD" in Hole Board test in mice.**

### 6.2.3. Defecation:

In that cases, there is significant result in defecation for total time periods for control, standard, 1X and 2X doses. So we cannot expect positive result from that.

**Table 5: Effect of XS 1x and XS 2x on the "D" in Hole Board test in mice.**

| Group | Number of "D" (Mean±SEM) | | | | |
|---|---|---|---|---|---|
| | -30 min | 30 min | 60 min | 120 min | 180 min |
| Control | 0.83±0.31 | 0.83±0.31 | 0.50±0.22 | 0.50±0.22 | 0.50±0.22 |
| STD (Benzodiazepum5mg/kg) | 0.33±0.21 | 0.33±0.21 | 1.83±0.60 | 1.50±0.62 | 0.33±0.21 |
| XS 1x | 1.17±0.41 | 0.17±0.17 | 0.83±0.48 | 0.50±0.22 | 0.67±0.21 |
| XS 2x | 1.17±0.67 | 1.50±0.56 | 1.83±0.70 | 1.0±0.52 | 1.17±0.48 |

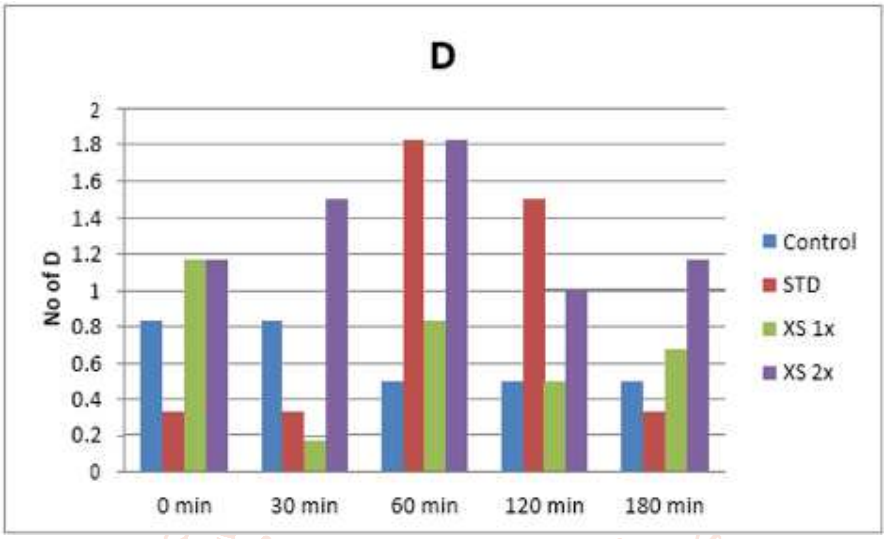


**Figure 6: Effect of XS 1x and XS 2x on the "D" in Hole Board test in mice**

### 6.3. Hole cross test:

The experiment was designed to find out whether the drugs under consideration have any effect on exploratory behavior or not. In that cases, there is also moderate significant result for total time periods for control, standard, 1X and 2X doses. So we can expect positive result from that.

**Table 6: Effect of XS 1x and XS 2x on the number of Hole Cross test in mice.**

| Group | Number of Hole Cross (Mean±SEM) | | | | |
|---|---|---|---|---|---|
| | -30 min | 30 min | 60 min | 120 min | 180 min |
| Control | 0.17±0.17 | 0.33±0.21 | 0.33±0.00 | 0.17±0.17 | 0.17±0.17 |
| STD (Benzodiazepum5mg/kg) | 0.17±0.17 | 0.50±0.34 | 2.37±1.76 | 1.50±1.31 | 0.56±0.00 |
| XS 1x | 0.33±0.21 | 1.0±0.52 | 1.67±0.67 | 1.83±0.87 | 1.17±0.54 |
| XS 2x | 0.17±0.17 | 0.17±0.17 | 1.0±0.82 | 1.0±1.0 | 0.67±0.67 |

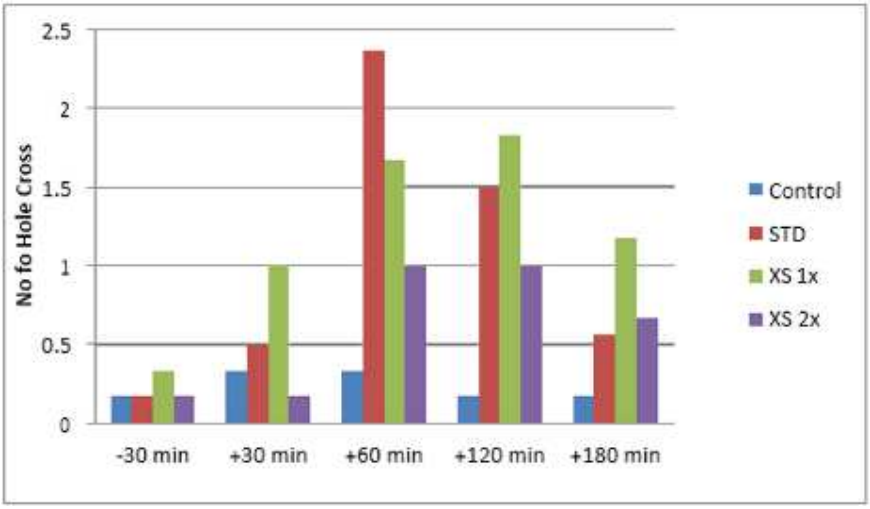


**Figure 7: Effect of XS 1x and XS 2x on the number of Hole Cross test in mice.**

### 6.4. Open field test

The test was designed in such a manner so that the test animal was not assigned to performed any specific task. it has been reported that in the absence of any specific task to perform, the behaviors of a given animal tends to maintain that inner activation level that is at precise time in consistence with the actual level of activation of the animal. The open field test was designed to assess the effect of any each substance on the exploratory activity of the animals.

#### 6.4.1. Center Tendency:

There is no significant result for control, standard, 1x and 2X doses during the total period of time.

**Table 7: Effect of XS 1x and XS 2x on the "Number of move to the center"in Open Field test in mice.**

| Group | Number of travel to center (Mean±SEM) | | | |
|---|---|---|---|---|
| | -30 min | 30 min | 60 min | 120 min |
| Control | 0.17±0.17 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| STD (Benzodiazepum5 mg/kg) | 0.00±0.00 | 0.17±0.17 | 0.50±0.34 | 0.00±0.00 |
| XS 1x | 0.00±0.00 | 0.00±0.00 | 0.33±0.33 | 0.33±0.33 |
| XS 2x | 0.17±0.17 | 0.00±0.00 | 0.17±0.17 | 0.00±0.00 |

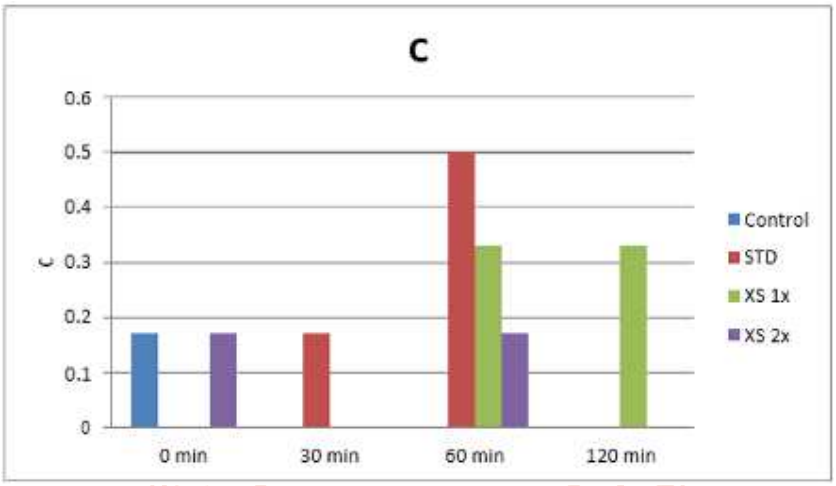


**Figure 8: Effect of XS 1x and XS 2x on the "C" in Open Field test in mice.**

#### 6.4.2. Stool:

There is significant result for control, standard, 1x and 2X doses during the total period of time.

**Table 8: Effect of XS 1x and XS 2x on the "S" in Open Field test in mice.**

| Group | Number of travel in "S" (Mean±SEM) | | | |
|---|---|---|---|---|
| | -30 min | 30 min | 60 min | 120 min |
| Control | 0.83±0.40 | 0.67±0.67 | 0.17±0.17 | 0.83±0.40 |
| STD (Benzodiazepum 5 mg/kg) | 1.0±0.63 | 0.67±0.67 | 0.83±0.31 | 0.00±0.00 |
| XS 1x | 1.17±0.48 | 1.17±0.60 | 0.83±0.66 | 0.33±0.33 |
| XS 2x | 0.67±0.50 | 0.67±0.33 | 0.33±0.21 | 0.00±0.00 |

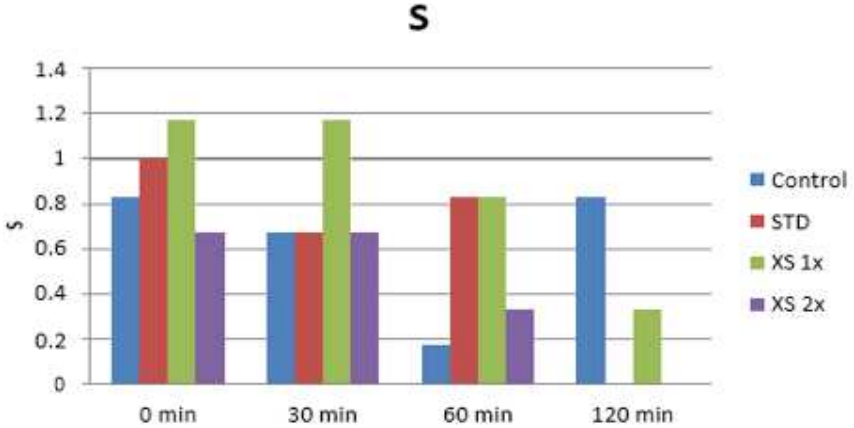


**Figure 9: Effect of XS 1x and XS 2x on the "S" in Open Field test in mice.**

### 6.4.3. Stand:

There is significant result for control, standard 1X and 2X doses during the total period oftime.

**Table 9: Effect of XS 1x and XS 2x on the "St" in Open Field test in mice.**

| Group | Number of travel in "St" (Mean±SEM) | | | |
|---|---|---|---|---|
| | -30 min | 30 min | 60 min | 120 min |
| Control | 3.17±0.54 | 3.17±0.48 | 2.50±0.57 | 2.67±0.50 |
| STD (Benzodiazepum5 mg/kg) | 2.67±0.33 | 5.00±0.63 | 4.17±0.75 | 4.17±0.66 |
| XS 1x | 2.33±0.21 | 4.83±0.48 | 3.67±0.21 | 3.83±0.48 |
| XS 2x | 3.17±0.31 | 3.50±0.56 | 3.50±0.76 | 3.17±0.48 |

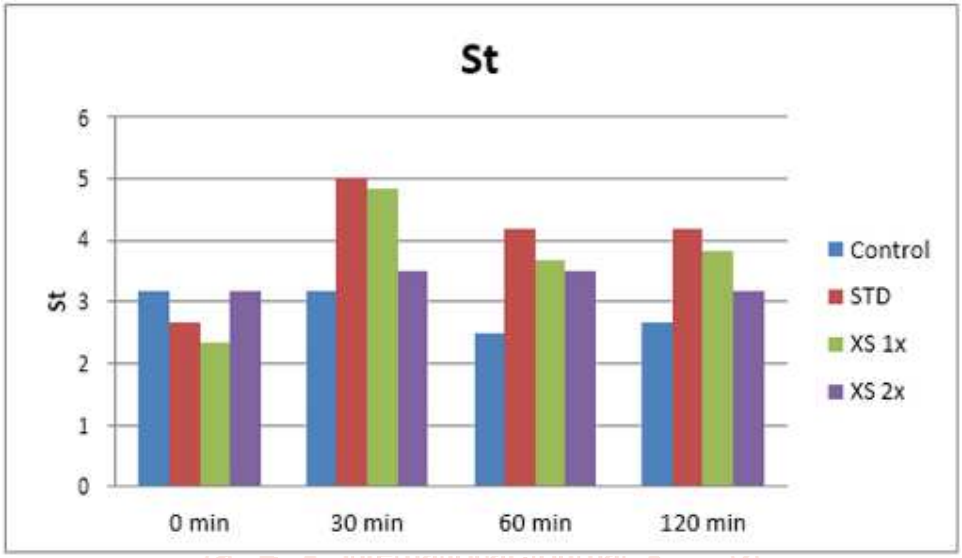


**Figure 10: Effect of XS 1x and XS 2x on the "St" in Open Field test in mice.**

### 6.4.4. Movement:

In that cases, there is also moderate significant result for total time periods for control, standard,1X and 2X doses. So we can expect positive result from that.

**Table 10: Effect of XS 1x and XS 2x on the square crossed in Open Field test in mice.**

| Group | Number of square crossed (Mean±SEM) | | | |
|---|---|---|---|---|
| | -30 min | 30 min | 60 min | 120 min |
| Control | 52.83±10.16 | 44.83±11.30 | 34.17±10.05 | 31.83±9.09 |
| STD (Benzodiazepum5 mg/kg) | 58.00±20.04 | 57.17±16.70 | 65.33±26.56 | 61.54±16.82 |
| XS 1x | 41.17±15.59 | 55.50±12.11 | 56.33±15.52 | 60.50±21.61 |
| XS 2x | 48.50±15.07 | 53.17±16.08 | 42.33±13.10 | 26.67±7.40 |

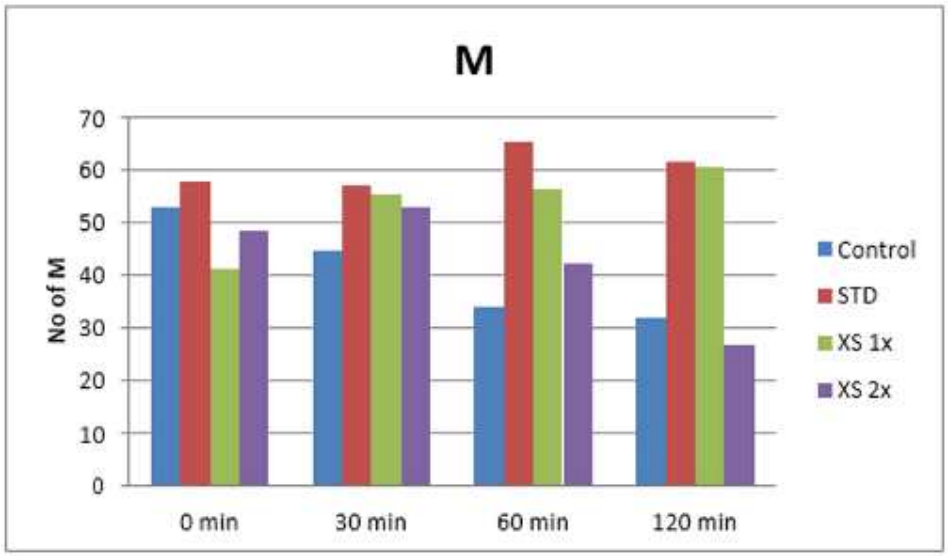


**Figure 11: Effect of XS 1x and XS 2x on the "M" in Open Field test in mice.**

## 7. DISCUSSION

X. strumarium has been widely used in clinical practise in various nations due to its anti-AR, anti-inflammatory, analgesic, and anti-tumor activities. In the meanwhile, numerous contemporary investigations into X. strumarium were also conducted, and its pharmacological functions and chemical make-up have been preliminary examined. However, it is still very important to figure out how to assure pharmaceutical safety, develop clinical efficacy of X. strumarium, and determine the mechanism of pharmacological activities and its related substances.

This study examined some neuropharmacological effects of X. strumarium and established that it has anxiogenic- and antidepressant-like activities. One of the most extensively used tests with the highest level of sensitivity to the effects of both anxiolytic and anxiogenic medications is the EPM[12]. Normal mice prefer to spend the majority of their allowed time in the closed arms during EPM tests. This predilection seems to reflect a dislike of open arms that is brought on by apprehensions about wide-open areas. Drugs like diazepam that promote open arm exploration are referred to as anxiolytics, while anxiogenics are the opposite[13]. In this study, we observed that the administration of different doses of test extract of X. stramonium induced an anxiogenic-like effect in mice, as it decreased open arm entries and the time spent in the open arms of the EPM when compared to the control animals. In addition, the study on locomotors activity, as measured by hole cross and open field tests, showed that both doses of extract from X. strumarium have significant effect on movements of treated mice.

## 8. FUTURE PERSPECTIVES AND CONCLUSION

Taking into account all the pharmacological data obtained, the results suggest that the *Xanthium stramorium* extract have significant effect on the CNS except in case of area exploration test. However, further studies will be necessary to clarify this more evidently. Plant science, in essence the constant increase in the scientific knowledge of plants and their chemical activity along with interaction with the environment, underpins modern plant science and agriculture. More study and research needed in that field.

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