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INVESTIGATION OF IN-VIVO ANALGESIC, ANTI-DIARRHEAL AND CNS ACTIVITY TEST OF ETHANOLIC EXTRACTS OF *Psychotria silhetensis* LEAVES

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Abstract

The importance of botanical, chemical, pharmacological evaluation of plant-derived agents used in the treatment of human ailments has been increasingly recognized in the last decades. Plants continue to be an important source of new drugs even today. P. silhetensis belonging to the family rubiaceae is a tree and has been used in traditional medicine to relief of various health problems such as cold, hepatitis, diarrhoea, heat stroke, dyspepsia, tuberculosis, sexual debility troubles, rheumatism and also important to promote intellect and enhancing memory, thus supporting its possible anti-Alzheimer's properties. This plant is really rare in Bangladesh (Hill tract plant), so most of the activities are not known. This study aims to evaluate the analgesic,anti-diarrheal & CNS activity test of P. silhetensis leaves.In this study, analgesic,anti-diarrheal & CNS effect of crude ethanolic extract of P. silhetensis plant were examined using swiss albino mice. We determined the analgesic activities of P. silhetensis by assessing acetic acid induced writhing, formalin induced paw licking, eddy's hot plate and tail immersion method. Castor oil induced method & charcoal meal GIT motility test was used to assess anti-diarrheal activity. We also determined the CNS activities by assessing often field method & hole cross method. The extract displayed good analgesic effect in acetic acid and formalin induced paw licking models. The extract prolonged the latency period to the thermal stimuli in both hot plate and tail immersion test.In the antidiarrheal tests, diarrheal suppression was highest at 200 mg/kg dose for the extracts, compared with loperamide in castor oil induced diarrhea model & diarrheal suppression was highest at 400 mg/kg dose for the extracts, compared with loperamide in charcoal meal GIT motility test. In often field method,the extract P. silhetensis showed the most effective depressant effect, for 200 mg/kg dose and number of movements for 200 mg/kg dose after 120 min , whereas in the hole cross test, all the extracts exhibited significant depressant effect in relation to positive control, diazepam. This study recommends that the methanolic extract of P. silhetensis shows good analgesic, anti-diarrheal, and CNS depressant properties.

Keywords: Anti-diarrheal, CNS depressant, analgesic, *Psychotria silhetensis* leaves, GIT motility.

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INTRODUCTION

The mankind has been a victim of diseases since the very beginning of their existence. But Mother Nature provided us with the remedy of those menacing Diseases. Despite the immense advances in modern medical science, still most of the people all around the world rely on medicinal plants for the purpose of treatment. According to WHO, about 80% of the world population, particularly the people living in the developing countries use various traditional medicines which rely on plants as sources of drugs [1]. It is absolutely imperative to ensure that these medicinal plants or their products really possess the claimed properties and exert the desired therapeutic effects. In an effort to substantiate the validity of claimed therapeutic effects of medicinal plants, they must be subjected to extensive scientific study. Attempts must be made to exclude the useless plants those are misleadingly claimed to be medicinal.

Medicinal plants can exhibit unwanted side effects due to the presence of some additional toxic constituents when used in the crude from [2]. So, the purpose of extensive phytochemical and pharmacological works is to isolate the active constituents in the pure form to avoid adverse effects and to ensure safe use of herbal drugs. Some of the compounds possessing a wide range of pharmacological activities are either impossible of difficult to synthesize in the laboratory. Many chemical compounds of diversified nature from plants often played an important role to give a new direction for laboratory synthesis of many new classes of drug molecules. In some cases, the plant components have become the starting material in the synthetic process of industrial production of many drug molecules.

There are numerous diseases in which any satisfactory treatment is yet to be discovered. Emergence of newer disease is also leading the scientists to go back to nature for newer effective molecules. Despite the recent interest in molecular modeling, combinatorial chemistry and other synthetic chemistry techniques by pharmaceutical companies, these compound libraries may not always be very diverse. Norman R. Farnsworth of the University of Illinois declared that, for every disease that affect mankind there is a treatment and cure occurring naturally on the earth. Medicinal plants are constantly getting more and more interest of the scientists due to their countless and extremely diverse chemical constituents from where scientists are hoping to find the answer to those diseases. Surprisingly, less than 15% of the plants are known to have been investigated pharmacologically out of the estimated 500,000 species of higher plants growing on earth [3]. Thus plants are considered as one of the most important and interesting subjects that

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should be explored for the discovery and development of newer and safer drug candidates. Bangladesh has a rich and prestigious heritage of herbal medicines among the South Asian countries. More than 500 species of medicinal plants are estimated as growing in Bangladesh and about 250 species of them are used for the preparation of traditional medicines. However, the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compounds [4]. Traditional records and ecological diversity indicate that Bangladeshi plants represent an exciting resource for possible lead structures in drug design [5].

Name of the plant

Psychotira silhetensis is one of few species of plants that are able to successfully be propagated via leaf cutting. Stem cuttings with *P. silhetensis* are also very easy and have the added benefit of startingto grow right away, making them far faster than leafcuttings [6].

Fig.1: *Psychotria silhetensis*

Scientific classification of *Psychotria silhetensis*

Kingdom:Plantae

Division:Magnoliophyta

Class:Magnoliopsida

Order:Gentianales

Family:Rubiaceae

Genus:Psychotria

Species: *Psychotria silhetensis*

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Constituents

Chemical analysis of dried *Psychotria silhetensis* has revealed that it contains roughly 0.10% to 0.66% alkaloids. About 99% of these alkaloids comprise dimethyltryptamine (DMT) - a controlled substance in many regions. In addition, it also contains other alkaloids like N-methyltryptamine (NMT) and beta-carbolines. It is said that the plants have the maximum alkaloid content during the morning [7].

Description of the plants

P. silhetensis is an evergreen shrub which can grow into a small tree with a woody trunk, but which usually remains at a height of 2-3 meters. It has long whorled, narrow leaves with a color ranging from light to dark green and a shiny top. The flowers have greenish white petals on long stalks. The fruit is a red berry containing many small long oval seeds. *P. silhetensis* is native to forests throughout the Amazon basin, and as far north as Central America and Cuba [8].

P. silhetensis is quite difficult to propagate from seeds, as they may require sixty days to germinate and sometimes as few as one in a hundred seeds will do so. Cultivation from cuttings is generally more successful – a small branch may simply be put in the ground and watered. Even pieces of branch containing only two leaves will often develop and grow successfully [9].

Effects of the *Psychotria silhetensis*

The main active compound in *P. silhetensis* is DMT (dimethyltryptamine). DMT may be smoked by itself, but psychedelic effects of pure DMT are very mild. *P. silhetensis* is in itself not active orally.

DMT is a serotonergic psychedelic, which means it achieves its effects by acting as strong partial agonists at the 5-HT2A receptors, elevating serotonin levels.

DMT is usually consumed in combination with a MAO inhibitor. The most commonly – and traditionally – used substance is Banisteriopsis caapi. Ayahuasca is still one of the most commonly used herbal concoctions used to induce psychedelic effects.

Ayahuasca has several physical, cognitive and visual effects. For most users, taking ayahuasca is a spiritual experience [10]. Hallucinations range from colourful geometric patterns to alien lifeforms.

One of the after effects of ayahuasca is an increased sense of mindfulness. This is thought to be the result of a purging of body and soul. Unfortunately, the purging of the body includes extensive vomiting and diarrhea [11].

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METHOD & MATERIALS

Analgesic activity test

Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain acts as a warning signal against disturbances of the body and has a proactive function. Analgesic means a drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. So, analgesic activity means capacity of a substance to neutralize the pain sensation. Any member of the group of drugs used to relieve pain (achieve analgesia) is known as analgesic. The word analgesic derives from Greek an- ("without") and algos ("pain"). So, the analgesic drugs are also known as painkiller [12].

Fig. 2: Swiss albino mice

Experimental animals

Sixteen experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III,and group-IV consisting of 4 mice in each group. Each group received a particular treatment i.e. control, standard and the dose of the extracts of the plant respectively. Prior to any treatment, each rat was weighed properly and were marked as M-1=Mouse 1, M-2=Mouse 2, M-3=Mouse 3,M-4=Mouse 4, and the dose of the test sample and control materials was adjusted accordingly.

• Group 1- Saline water

• Group II - Standard (Indomethacin)

• Group III - 100mg of Psychotria Sylhetensis plant

• Group IV-200mg of Psychotria Sylhetensis plant

Counting of Writhing

Each mice of all groups were observed individually for counting the number of writhing they made in 30 minutes commencing just 5 minutes after the intraperitoneal administration of acetic

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acid solution. The animal did not always accomplish full writhing, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly two half writhing were taken as one full writhing.

Eddy's hot plate method

In this method, the experimental animal is placed on the hot plate at 55˚C. As a result, Mice will lick the paw or jump from the hot plate. Any substance that has got analgesic activity is supposed to lessen the paw licking or jumping of animals within in a given time frame and with respect to the control group. The paw licking or jumping inhibition of positive control was taken as standard and compared with test samples and control. As positive control, any standard NSAID drug can be used. In the present study, indomethacin was used to serve the purpose.

Counting of Licking or jumping

Each mice of all groups were observed individually for counting the number of licking or jumping they made at 0, 0.5,1,1.5,2 and 2.5 hour after the respective treatment

Tail immersion method

In this method, the tip of tail of the mice is dipped up to 5 cm in hot water maintained at 55˚C. As a result, mice will withdraw its tail from the hot water and it is noted as the reaction time. Any substance that has got analgesic activity is supposed to lessen the withdrawal time within in a given time frame and with respect to the control group. The withdrawal time inhibition of positive control was taken as standard and compared with test samples and control. As positive control, any standard NSAID drug can be used. In the present study, Indomethacin was used to serve the purpose.

Evaluation of Anti-Diarrheal property

Intestinal diseases are one of the main causes of death of infants particularly in developing countries WHO;1994 It thus becomes important to identify and evaluate commonly available natural drugs as alternative to currently used anti diarrhea drugs.

We can determine the Anti-diarrheal activity by following method:

- Castor oil induced method
- Charcoal meal Gastrointestinal motility Tests

Charcoal meal Gastrointestinal motility Tests

In the Gastrointestinal motility experiment, there was also a dose dependent reduction in the length of the intestine travelled by the charcoal meal. The percentage inhibition in all the doses

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was significantly higher when compared with the negative control group. One ml of charcoal meal (10% charcoal suspension in 5% gum acacia) was administered orally 30 minutes after the treatment. The rats were sacrificed after 1h and the distance travelled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum [12].

Determination of CNS Activity

Sedative and hypnotics are the drugs which can reduce anxiety and produce a calming effect by inducing the onset of sleep as well as maintaining sleeping duration.Nowadays, these drugs are extensively used in treatment of different psychiatric disorders including anxiety and insomnia. Thus, development of new sedative-hypnotic drugs with fewer side effects has been suggested to be a promising approach to combat different psychiatric disorders [13]. We can determine the CNS Activity of *P.silhetensis* by the following method:

- Open field method
- Hole cross method
- Forced swimming test
- Rota-Rod Test

RESULT AND DISCUSSION

Result of analgesic activity test of *Psychotria silhetensis*

Acetic acid induced writhing method

In acetic acid-induced writhing inhibition test, the ethanol extract of *P. silhetensis* at both doses (100 and 200 mg/kg body weight) showed good analgesic activity. The percentage of inhibitions of the writhing response at the doses 100 mg/kg and 200 mg/kg were 24.52% and 33.96%, respectively which was comparable with the positive control Indomethacin (49.05%).

Table 1: Effect of ethanolic extracts of P. silhetensis leaves by acetic acid induced writhing in mice.

Groups	Treatment	Dose	No. of writhing (Mean ± SEM)	% Writhing inhibition
Group-I (Control)	Saline water	0.5 ml/10gm body weight	13.25± 1.72	--
Group-II (Standard)	Indomethacin	10 mg/kg body weight	6.75± 0.98	49.05
Group-III	Plant extract	100 mg/kg body weight	10 ± 0.81	24.52
Group-IV	Plant extract	200 mg/kg body weight	8.75 ± 2.22	33.96

Values are expressed as mean ±S.E.M.

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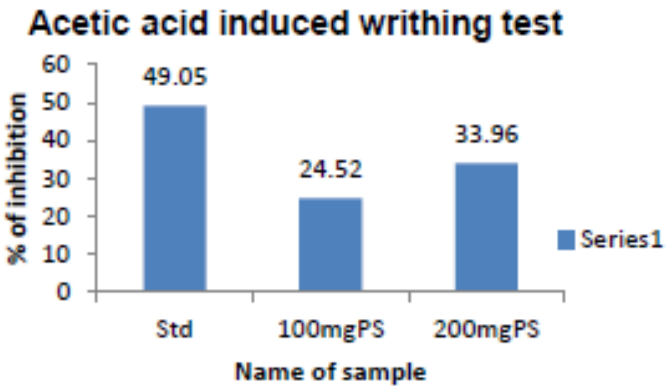

Fig. 3: Graphical Representatin of analgesic activity *P. silhetensis* extract Compared to Standard

Formalin Induced Paw Licking Test

The result of analgesic activity of *P. silhetensis* leaves is shown at the table-02.The ethanolic extract of *P.silhetensis* leaves at the dose 100 mg/kg and 200 mg/kg b.w showed analgesic effect compared to control on formalin induced paw licking in mice.

Table 2: Effect of ethanolic extracts of P. silhetensis leaves by formalin induced paw licking in mice.

Groups	Dose	First 5 min	Inhibition (%)	20-30 min	Inhibition (%)
Control	-	15.75±1.19	-	10.75±0.98	-
Indomethacin	10	9.25±0.98	41.27	5.75±0.55	46.57
Plant extract	100	11.75±0.55	25.39	7±1.24	34.88
Plant extract	200	11.25±1.28	28.57	6±1.49	44.18

Values are expressed as mean ±S.E.M.

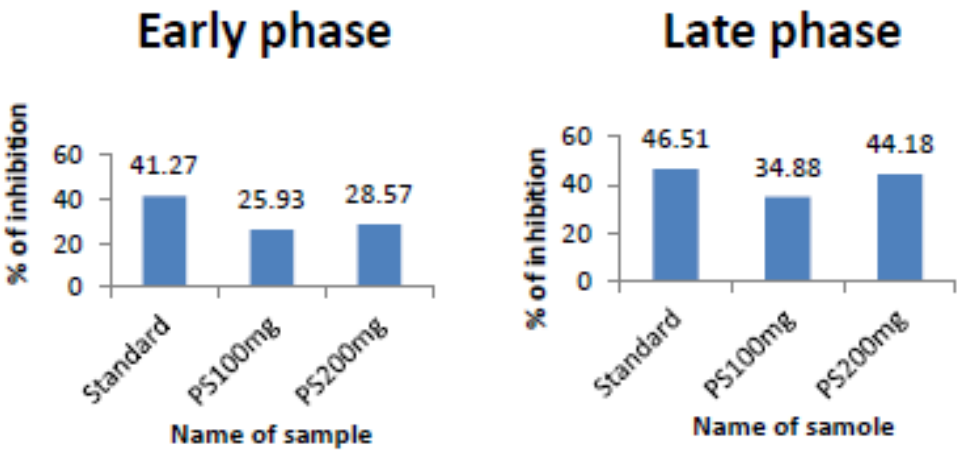

Fig. 4: Time spent in licking for different groups in early phase & late phase

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Result of Eddy's hot plate method

In the hot plate method the extracts exhibited potent analgesic activity. The result of analgesic activity of *P. silhetensis* leaves is shown at the table 3.The ethanolic extract of *P.silhetensis* leaves at the dose 100 mg/kg and 200mg/kg b.w. showed analgesic effect compared to control on Eddy's hot plate in mice.

Table 3: Effect of ethanolic extracts of *P. silhetensis* leaves by eddy's hot plate in mice

Treatment	Reaction time in hours					
	0hr	0.5hr	1hr	1.5hr	2hr	2.5hr
Control	6.5±1.10	5±0.94	7.25±0.98	3.5±1.45	5.25±1.65	5.75±1.36
Standard	2.5±0.33	2.25±0.55	3.25±0.55	1.75±0.28	2.75±0.28	2.5±0.33
Extract (100mg/kg)	3.5±0.74	2.75±0.28	4.25±0.28	2.25±0.28	3.5±0.33	3.5±0.74
Extract (200mg/kg)	3±0.47	2.25±0.98	3.25±0.55	2±0.47	3±0.47	3.25±0.72

Values are expressed as mean ±S.E.M.

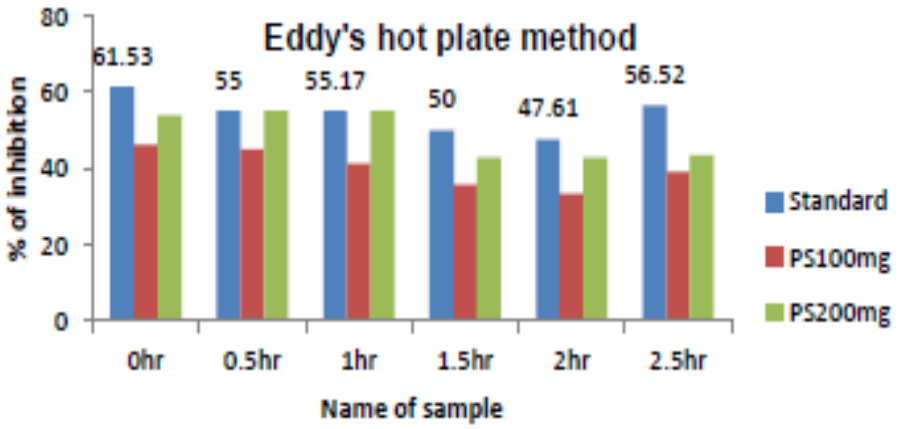

Fig. 5: Graphical Representation of analgesic activity *P. silhetensis* extract Compared to Standard

Result of tail immersion method

It is a thermal method, done to evaluate the anti-nociceptive effect of *Psychotria silhtensis* leaves. The result of analgesic activity of *P. silhetensis* leaves is shown at the table-03.The ethanolic extract of *P.silhetensis* leaves at the dose 100 mg/kg and 200 mg/kg b.w showed analgesic effect compared to control on tail immersion in mice.

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Table 4: Effect of ethanolic extracts of P. silhetensis leaves by tail immersion method in mice

Treatment	Reaction time in hours					
	0hr	0.5hr	1hr	1.5hr	2hr	2.5hr
Control	3.25±0.55	6.25±0.55	4.75±0.98	5.5±0.74	7.75±0.28	7.75±0.28
Standard	1.75±0.55	3±0.47	2±0.47	1.75±0.28	2.25±0.55	2±0.47
Extract (100mg/kg)	2.25±0.28	3.25±1.36	3±0.47	3.25±0.55	3.75±0.47	4±0.81
Extract (200mg/kg)	2±0.47	2.75±0.98	2.5±0.33	3±0.47	3.75±0.86	4±0

Values are expressed as mean ±S.E.M.

Discussion on Analgesic activity test

In the analgesic activity performed using acetic acid-induced writhing,formalin induced paw licking,Eddy's hot plate,and tail immersion inhibition test in mice, the inhibition increased as the dose of extract was increased . Preliminary qualitative phytochemical screening reveals the presence of alkaloids, flavonoids, steroids, and tannins in *P*.silhetensis flavonods, tannins, and alkaloids have been reported to have a role in analgesic activity primarily by targeting prostaglandins Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response.Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipid via cyclooxygenase & prostaglandin biosynthesis. The studied plant (*P. silhetensis*) showed good pain inhibition compared to control group. In formalin induce paw licking method *P. silhetensis* reduces inflammatory pain 0-5 minutes and 20-30 minutes compared to control group. In Eddy's hot plate and tail immersion method *P. silhetensis* starts 0-2.5hr and shows maximum analgesic activity compared to standar group. So the result found in the present study demonstrated that the leaf extracrs of *P.silhetensis* has good analgesic effect.

Anti-diarrheal test

Method-1: Castor oil induced method

In castor oil-induced diarrhea inhibition test, *P silhetensis* leaves extract at the dose of 100 mg/kg and 200 mg/kgb b.w. showed table-05 an increase in mean latent period for diarrhea episode. However, the extract at both doses decrease in mean number of stools and total weight of fecal output compared to control group.

Method-2: Charcoal meal GIT motility test

In this method, the mean distance travelled by the charcoal meal in small intestine and intestinal motility was measured as compared with control group. The ethanolic extract of *P. silhetensis*

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leaves at the doses 200 mg/kg and 400 mg/kg body weight showed highly anti-diarrheal effects compared to control by Charcoal meal GIT motility test. These doses also reduced the mean distance travelled by charcoal and there was significant difference as compared to control.

Discussion on Ant- diarrheal activity test

Castor oil induced diarrhea model is widely used for the evaluation of anti- diarrheal property of drugs.Ricinoleic acid,the active metabolite of ricinoleic acid which is present in castor oil is responsible for the diarrhea inducing property castor oil.It stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestine mucosa.Its action also stimulates the release of endogenous prostaglandin which stimulates motility and secretion.Evaluation of anti-diarrheal activity of *P. silhetensis* shows anti-diarrheal activity compared to control group.That means *P.silhetensis* showed maximum anti-diarrheal activity.

Charcoal meal GIT motility test model is widely used for the evaluation of anti- diarrheal property of drugs. Evaluation of anti-diarrheal activity of *P. silhetensis* shows anti-diarrheal activity compared to control group. Preliminary qualitative phytochemical screening of *P. silhetensis* reveals that the plant is a rich source of alkaloids, flavonoids, steroids, and tannins which justify the excellent anti-diarrheal effect of the plant extract.

Result of CNS method

Method-1: Often field method

Results of the open-field test of *P. silhetensis* is given Table-07. The *P.silhetensis* extract exhibited a decrease in the movements of the test animals at dose 200 mg/kg and 400 mg/kg. The results were statistically good for both doses at 30 min, 60min, 90min & 120min.

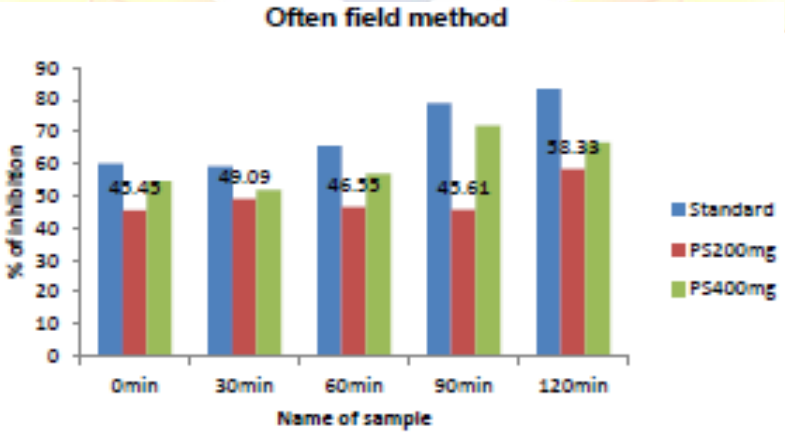

Fig. 6: Graphical Representation of CNS activity of P. silhetensis extract Compared to Standard

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Method-2: Hole cross Method

Results of the hole-cross test of P. silhetensis is given in Table-06. They were statistically good for dose 200 mg/kg and 400 mg/kg after 30, 60, 90 and 120 minute compared to control.

Table 5: Effect of ethanolic extracts of P. silhetensis leaves by hole cross method in mice

Treatment	Reaction time in hours				
	0 min	30 min	60 min	90 min	120 min
Control	12.5±2.42	10±0.81	11±0.81	7.25±0.98	5.75±1.28
Standard	3±1.41	4.75±0.72	6.5±0.57	3.75±0.86	1.75±1.36
Extract (100mg/kg)	5.25±0.98	6.25±0.55	4±0.47	4±0.81	3.75±0.55
Extract (200mg/kg)	4.25±0.55	5.55±0.57	6.5±0.74	4±0.47	2.75±0.55

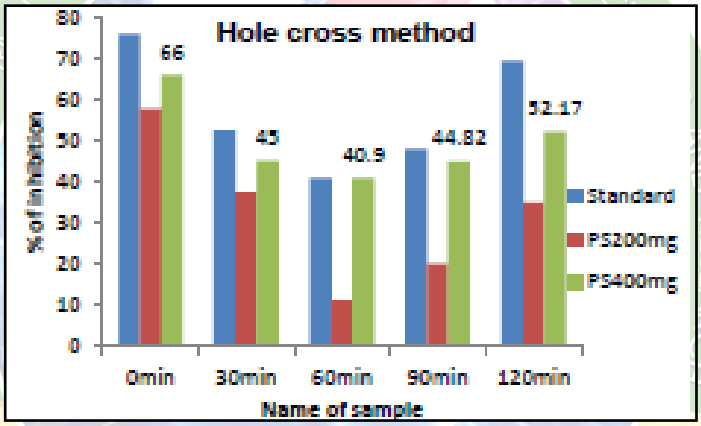

Fig. 7: Graphical Representation of CNS activity *P. silhetensis* extract Compared to Standard

Discussion on CNS activity test

The open field test is utilized to evaluate the emotional state of animals. Thus, animals removed from their acclimatized cage and placed in a novel environment express anxiety and fear by showing alteration in all or some parameters, such as decreases in ambulation and exploration, immobilization or ‘‘freezing’’, reduction in normal rearing and in grooming behavior, and increased maturation and defecation due to augmented autonomic activity.The extracts *P. silhetensis* produced sedative effect due to the good inhibition in the number of central squares crossed. In hole cross method Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. CNS depressant drugs mainly exert their action through GABAA receptor. Therefore, the sedative effect of the extract at both doses may be due to hyperpolarization of the CNS via interaction with GABAA or benzodiazepine receptor. Further

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studies are needed to evaluate this. In addition to the sedative effect, the decrease in movement may be due to the muscle relaxant effect of the plant extract. The decrease in locomotion activity by diazepam treated mice compare with the control group.

CONCLUSION

Based on the results of our study it can be concluded that the etanolic extract of *Psychotria silhetensis* leaves possess moderately inhibit analgesia in different pain models done for analgesic activity. The present study supports about the use of the leaves extract of *P. silhetensis* in the treatment of diarrhea because it sufficiently inhibit diarrhea.

The result of the present study indicates the ethanolic extract of *P. silhetensis* leaves shows CNS activity evaluated by open field and hole cross methods in mice.

All of the experiment were performed in multiple dose and further studies have to be carried out know its constituents responsible for activity and to evaluate other therapeutic effects.

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