EVALUATION OF ANTIPSYCHOTIC EFFECT OF ALLIUM CEPA

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ABSTRACT

Objective: To evaluate the antipsychotic effect of Allium cepa.

Materials and Methods: The antipsychotic effect of the Allium cepa was evaluated on haloperidol induced catalepsy, cooks pole climbing apparatus, locomotor activity on actophotometer, ketamine induced stereotype behavior. Different groups of rats were fed orally with a specially prepared diet containing various concentrations of Allium cepa paste (ACP) for 30 days consecutively. Further, the biochemical estimations were done by estimating brain dopamine levels. Results: The ACP produced significant dose dependent potentiation of haloperidol (1 mg/kg, i.p.) induced catalepsy in rats, significantly increased the time taken by the rat to climb the pole in dose dependent manner, significantly decreased the locomotor activity. The ACP significantly decreased ketamine (50 mg/kg, i.p.) induced stereotyped behavior in a dose dependent manner. ACP significantly decreased the brain dopamine level. Conclusion: The results suggest that ACP poss’es antipsychotic activity. Further neurochemical investigation can explore the mechanism of action of the plant drug with respect to anti-dopaminergic and anti serotoninergergic functions and help to establish the plant as an antipsychotic agent.

KEY WORDS: Anti-dopaminergic, catalepsy, stereotypy, ketamine.

INTRODUCTION

Herbs are nature’s gift to mankind and an herbal renaissance is blooming across the world. Medicinal herbs contain substances known to modern and ancient civilizations for their healing properties. They have been important to mankind, both socially and economically, for...
thousands of years. Onion *Allium cepa*, belongs to family lilliaceae, shows only a single vertical shoot above the ground and is used for energy storage and as species; it could be in various shapes and sizes. It is distributed throughout temperate regions of the world including Europe, Asia, North America and Africa.\(^1\) Phytochemicals present in onion include phenolic acid (caffeic, sinapic, p-coumaric, protocatechuic acids), flavonoids (quercetin, isorhamnetin, taxifolin and their glycosides), anthocyanins (cyaniding, carboxypyranocyanidine and peonidin glycosides), sterols (cholesterol, stigmasterol, beta-sitosterol), saponins (tropeosdies and ascalonicosides), vitamins (A, C, B and B2), pectin and peptides.\(^2\) The lachrymating principle in crushed or cut onion is thiopropanal S-oxide (propanethial S-oxide) produced from its precursor, trans-S-(1-propenyl)-cysteine sulfoxide, by the action of allinase. The fleshy bulb that grows below the ground is used medicinally as well as for food. The main biological properties of onion include antihypercholesterolemic, hypoglycemic, antimicrobial activity, antibacterial, antifungal, antiplatelets activity, antispasmodic activity, antidermatopytic action, cardiovascular support, antioxidant, anticancer effect, and asthma protection.\(^3\) Although several medicinal uses have been reported for ACP, no investigative report pertaining to its central nervous system activity exists. Hence, an attempt has been made to evaluate the antipsychotic activity of the *Allium cepa*.

**MATERIALS AND METHODS**

The of *Allium cepa* were purchased during the months of August, 2012 from local market of Hisar, Haryana and got authenticated from Raw Materials Herbarium & Museum, Delhi (RHMD)- (Ref. NSICAIR/RHDM/2014/2519/98-3). *Allium cepa* were ground into a fine paste using an electric grinder. Different concentrations of ACP (10, 15, 20% w/w) were fed to separate groups of rats and mice through a specially prepared diet. This special diet comprised of a mixture of *Allium cepa* paste, wheat flour kneaded with water, a small amount of refined vegetable oil and a pinch of salt (sodium chloride), to impart taste. Each rat consumed around 12gm/day and mice consumed around 3mg/day of this specially prepared diet. Control animals received the normal diet consisting of wheat flour, kneaded with water, small amount of refined vegetable oil and a pinch of salt but without ACP paste. The concentrations of ACP paste in diet were determined on the basis of pilot study, acceptability by the animals and literature reports.\(^4,5\)

**Animals:** Male Wistar rats (180- 220 g) and albino mice (25-30g) were used for the study. The animals were housed in colony cages and maintained under the standard environmental conditions.
Kadian et al. World Journal of Pharmacy and Pharmaceutical Sciences

conditions - temperature 25 ± 2°C, 12 h light: 12 h dark cycle and 50 ± 5 % relative humidity, with food and water ad libitum. All experiments were carried out during the light period (08.00 -16.00 h). The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 1538).

Drugs
All the drug solutions viz. Haloperidol (RPG Science Pharmaceutical Pvt. Ltd), Chlorpromazine, Ketamine and dopamine (Neon Pharmaceutical Pvt. Ltd) in the form of injections were purchased locally from retail chemists, Hisar were prepared in distilled water.

Haloperidol-induced catalepsy
Haloperidol (1 mg/kg) was injected i.p. on the 31th day to control rats (n = 6) treated with normal diet consisting of wheat flour, kneaded with water, small amount of refined vegetable oil and a pinch of salt but without ACP paste and to the rat fed with Different concentrations of ACP (10, 15, 20% w/w) through a specially prepared diet. The duration of catalepsy was measured at 0, 60, 90, 120, 150 and 180 min, using Bar test. Both the forepaws of mouse were placed on a horizontal bar raised 3 cm from the table, and the time required to remove the forepaws from the bar was recorded as the duration of catalepsy. In all the experiments, the observer was blind to the treatment given to the mice. Between experiments, the animals were returned to their home cages. [6]

Cooks Pole Climbing Apparatus
The pole-climb avoidance paradigm is an avoidance escape procedure used to separate neuroleptics from sedatives and anxiolytics. Whereas sedative compounds suppress both avoidance and escape responding at approximately the same doses, neuroleptic drugs reduce avoidance responding at lower doses than those affecting escape responding. Male Wistar rats weighing 150 gm were used in the training and testing of rat was conducted in the Pole climbing apparatus, which has a floor that acts as a source of shock. In the centre of the roof there is a wooden pole. The animals were trained as follows. Press the buzzer, Shock of 20v was delivered to the floor grid. The animal was trained to climb the pole to avoid shock. This was repeated until the animals learned to climb the pole soon after hearing the buzzer even without receiving the shock. Such rats, which climb the pole within 3 sec after pressing the buzzer, were chosen for this study. The training and testing of the rats is conducted in a 25
cm x 25 cm x 40 cm chamber that is enclosed in a dimly lit, sound-attenuating box. Scrambled shock is delivered to the grid floor of the chamber. A 2.8-kHz speaker and a 28-V light are situated on top of the chamber. The conditioning stimulus is presented alone for 4s and then is coincident with the unconditioned stimulus, a scrambled shock delivered to the grid floor, for 26s. The shock current is maintained at 1.5mA. A pole climb response during the conditioned stimulus period terminates the conditioned stimulus and the subsequent conditioned and unconditioned stimuli. This is considered an avoidance response. A response during the time when both the conditioned and unconditioned stimuli are present terminates both stimuli and is considered an escape response. Test sessions consist of 20 trials or 60 min, whichever comes first. There is a minimum intertrial interval of 90s. Any time remaining in the 30s allotted to make the pole climb is added to the 90s intertrial interval. Responses during this time have no scheduled consequences; however, rats having greater than 10 intertrial interval responses should not be used in the experiment. Before testing experimental compounds, rats are required to make at least 80% avoidance responses without any escape failures. Data are expressed in terms of the number of avoidance and escape failures relative to the respective vehicle control data. [6]

**Ketamine-Induced Stereotypic Behavior in Mice**

In this model animals were divided into five groups and each group consisted of six animals. The control animals received normal diet consisting of wheat flour, kneaded with water, small amount of refined vegetable oil and a pinch of salt but without $ACP$ and treated with Ketamine (50mg/kg, i.p) for 15 consecutive days. The animals of standard group received Haloperidol (1mg/kg, i.p) and after 30 min Ketamine was given, (50mg/kg, i.p) for 15 consecutive days. The animals of test groups received different concentrations of $ACP$ (10, 15, 20% w/w) through a specially prepared diet and after 30 min Ketamine was given (50mg/kg, i.p) for 15 consecutive days. Each mouse was individually placed into plastic cages ($37 \times 24 \times 30$ cm$^3$) divided into quadrants by lines on the floor and allowed to acclimatize for at least 30 min before the testing began. Behavioural tests were performed between 10 a.m. and 4 p.m. The stereotypic behaviour was assessed by counting the number of turning, weaving, and head bobbing. Turning was measured by counting turn around every 10 min over 60 min. Weaving and Head-bobbing were measured by counting its neck wave right and left, and go up and down every 10 min over 60 min. [7,8]
Locomotor Activity: The locomotor activity of rats was measured using Photoactometer (INCO, Ambala, India)\(^9\).

Biochemical Estimation
The animals were sacrificed by cervical dislocation, whole brain was rapidly frozen at -5°C and brain Dopamine level was spectrofluorimetrically estimated by the methods of Ansell and Beeson\(^{10}\) as modified by Cox and Perhach\(^{11}\).

Statistical analysis
Results are expressed as mean ± S.E.M. and the statistical analysis of data was done using one-way analysis of variance (ANOVA), followed by Dunnett’s test. Probability level less than 0.05 was considered statistically significant.

RESULTS
Haloperidol-induced catalepsy
In control animals, haloperidol (1 mg/kg, i.p.) produced the maximum catalepsy at 120 min (235 ± 5.275s). ACP (10, 15, 20% w/w) through a specially prepared diet, significantly potentiated haloperidol induced catalepsy at each time interval, in a dose dependent manner. At dose 10, 15, 20% w/w, ACP showed maximum cataleptic score 257 ± 9.998, 265 ± 5.852 and 278 ± 5.288s, respectively at 120 min (P < 0.01) in haloperidol treated animals.

![Fig 1. Effect of ACP on Haloperidol Induced Catalepsy of Rats](image)

ACP= *Allium cepa* paste (10, 15 and 20%w/w) were fed to separate groups of rats through a specially prepared diet.
CPZ= Chlorpromazine(10mg/kg, i.p) was dissolved in normal saline.
Values are in mean ± SEM (n = 6).
One way ANOVA followed by Dunnett’s t-test.
* denotes p<0.01 as compared to control group
# denotes p<0.05 as compared to control

Cooks Pole Climbing Apparatus: Administration of ACP 10% w/w, through a specially prepared diet for 30 successive days markedly (p<0.05) inhibited the conditioned avoidance response in rats as indicated by increased time spent on the grid floor of the chamber. However, the concentrations of 15 and 20% w/w of ACP were remarkably (p<0.01) effective in inhibiting the conditioned avoidance response. The effect of ACP was found to be comparable to that of Chlorpromazine (10 mg/kg i.p.) (Antipsychotic agents).

ACP= *Allium cepa* paste (10, 15 and 20%w/w) were fed to separate groups of rats through a specially prepared diet.
CPZ= Chlorpromazine(10mg/kg, i.p) was dissolved in normal saline.
Values are in mean ± SEM (n = 6).
One way ANOVA followed by Dunnett’s t-test.
* denotes p<0.01 as compared to control group
# denotes p<0.05 as compared to control group
Ketamine-Induced Stereotypic Behavior in Mice

Ketamine (50mg/kg, i.p) produced stereotypic behavior in mice. Different concentrations of ACP (10, 15, 20% w/w) through a specially prepared diet for 30 successive days remarkably (p<0.01) decreased this stereotypic behavior of mice produced by ketamine. Animals treated with Haloperidol (1mg/kg, i.p) reversed stereotypic behavior induced by ketamine. The effect of ACP was found to be comparable to that of Haloperidol (Antipsychotic agents).

Turning Behavior of Mice

Turning pattern was measured by counting the turn-around behavior of each mouse every 10 min over 60 min periods. Administration of ACP at the concentration of 10% w/w for 30 successive days showed no significant (p>0.05) effect on turning pattern during first 30 min periods. However, at the concentration of 15 and 20%w/w ACP remarkably (p<0.01) decreased the turning pattern of mice induced by ketamine. At the concentration of 10, 15 and 20%w/w showed no significant (p>0.05) reduction at 60 min. Animals treated with Haloperidol (1mg/kg, i.p) decreased the turning pattern.

![Fig 3. Effect of ACP on Turning Behavior of Mice](image)

ACP= *Allium cepa* paste (10, 15 and 20%w/w) were fed to separate groups of rats through a specially prepared diet.

Ket= ketamine (50mg/kg, i.p), Halo= haloperidol (1mg/kg, i.p), were dissolved in normal saline.

Values are in mean ± SEM (n = 6).

One way ANOVA followed by Dunnett’s t-test.

* denotes p<0.01 as compared to control group.
Weaving Behavior of Mice

Weaving pattern was measured by counting its paw movements standing on hind legs every 10 min over 60 min period. At first 10 min period concentration of 10, 15 and 20% w/w ACP remarkably (p<0.01) decreased weaving pattern of mice produced by ketamine. During 20 min and 30 min ACP 10% w/w showed no significant decrease in weaving pattern. However 15% and 20% w/w remarkably (p<0.01) decreased weaving pattern at 20 min. At 30 min concentration of 15% w/w ACP showed a remarkable (p<0.05) and 20% w/w remarkable (p<0.01) decrease in weaving pattern. At 60 min 20% w/w ACP remarkably (p<0.01) decreased the weaving behaviour and 10 and 15% w/w ACP showed no significant effect as compared to ketamine group. The effect of ACP was found to be comparable to that of Haloperidol (Antipsychotic agents).

ACP= Allium cepa paste (10, 15 and 20% w/w) were fed to separate groups of rats through a specially prepared diet.
Ket= ketamine (50mg/kg, i.p), Halo= haloperidol (1mg/kg, i.p), were dissolved in normal saline.

Values are in mean ± SEM (n = 6).

One way ANOVA followed by Dunnett’s t-test.
* denotes p<0.01 as compared to control group
# denotes p<0.05 as compared to control group
Head-Bobbing Behavior of Mice: Head-bobbing pattern was measured by counting its neck movements towards right and left and up and down every 10 min over 60 min period. Administration of ACP at the concentration of 10, 15 and 20% w/w for 30 successive days remarkably (p<0.01) reduced Head-bobbing pattern of mice produced by ketamine.

![Graph showing effect of ACP on head-bobbing behavior of mice](image)

Fig 5. Effect of ACP on Head-Bobbing Behavior of Mice

ACP = *Allium cepa* paste (10, 15 and 20% w/w) were fed to separate groups of rats through a specially prepared diet. Ket = ketamine (50mg/kg, i.p), Halo = haloperidol (1mg/kg, i.p), were dissolved in normal saline.

Values are in mean ± SEM (n = 6). One way ANOVA followed by Dunnett’s t-test.

* denotes p<0.01 as compared to control group
# denotes p<0.05 as compared to control group

Locomotor Activity: Administration of ACP 10% w/w, through a specially prepared diet for 30 successive days markedly (p<0.05) decreased Locomotor activity in rats measured using actophotometer. However, the concentrations of 15 and 20% w/w of ACP were remarkably (p<0.01) effective in decreasing Locomotor activity. The effect of ACP was found to be comparable to that of Chlorpromazine (Antipsychotic agents).
ACP= *Allium cepa* paste (10, 15 and 20%w/w) were fed to separate groups of rats through a specially prepared diet.

CPZ= Chlorpromazine(10mg/kg, i.p) was dissolved in normal saline.

Values are in mean ± SEM (n = 6).

One way ANOVA followed by Dunnett’s t-test.

* denotes p<0.01 as compared to control group

# denotes p<0.05 as compared to control group

**Brain Dopamine Level**

Administration of *ACP* at the concentration of 10% w/w for 30 consecutive days showed markedly significant (p<0.05) effect on brain dopamine level. However, administration of *ACP* at the concentration of 15 and 20%w/w for 30 consecutive days showed remarkably significant (p<0.01) decrease in brain dopamine level in rats compared to control group.
ACP= *Allium cepa* paste (10, 15 and 20%w/w) were fed to separate groups of rats through a specially prepared diet.
CPZ= Chlorpromazine(10mg/kg, i.p) was dissolved in normal saline.
Values are in mean ± SEM (n = 6).
One way ANOVA followed by Dunnett’s t-test.
* denotes p<0.01 as compared to control group
# denotes p<0.05 as compared to control group

**DISCUSSION**

Schizophrenia continues to be a mysterious disease fascinating the minds of psychiatrists, pharmacologists and neuroscientists all over the world for more than a century. The crucial welfare of the millions afflicted with schizophrenia is at stake. The cause of schizophrenia is not yet identified. However, it appears from the available reports that schizophrenia results from genetic, occupational and environmental risk factors, which act independently or combine synergistically to develop schizophrenia. In any case, schizophrenia should not be confined to split personality or multiple personality- disorder. Typically, a schizophrenic patient shows both, positive symptoms such as delusions, hallucinations or cognitive dysfunction and negative symptoms such as social withdrawal, inability to articulate or loss of emotional tone. Positive symptoms refer to a loss of contact with reality and comprise of hallucinations delusions, bizarre behavior and positive formal thought disorders. Negative symptoms refer to a diminution in or absence of normal behaviors and include flat affect, alogia, avolition and anhedonia. Cognitive symptoms manifest as deficits in attention, learning, memory, concentration and executive functions (abstract thinking, problem solving). In the present study, we have focused upon the effects of *Allium cepa* paste on psychosis. Phytochemicals present in onion include phenolic acid (caffeic, sinapic, p-coumaric and protocatechuic acids), flavonoids (quercetin, isorhamnetin, taxifolin and their glucosides), anthocyanins (cyaniding, carboxyppyranocyanidine and peonidin glycosides), sterols (cholesterol, stigmasterol, beta-sitosterol), saponins (tropeosdies and ascalonicosides), vitamins (A, C, B and B2), pectin and peptides. Experimental studies have shown that phenolic compounds particularly flavonoids and vitamins, present in ACP are important antioxidants and superoxide scavengers. The antioxidant activity of ACP may be responsible for its beneficial antipsychotic action. The therapeutic and pharmacological actions of Onion (antihypercholesterolemic, hypoglycemic, antimicrobial activity, antibacterial, antifungal, antiplatelets activity, antispasmodic activity, antidermatopytic action, cardiovascular support, antioxidant, anticancer effect) are noteworthy Haloperidol, a typical neuroleptic produces catalepsy in rodents and extrapyramidal side effects in human [12]. Haloperidol-induced
catalepsy is one of the animal models for testing the extrapyramidal side effects of antipsychotic drugs. Haloperidol, (a non-selective D2 dopamine antagonist) induced catalepsy is primarily due to blockade of dopamine receptors in the striatum. The striatum and nucleus accumbens have been implicated as the major brain structures involved in antipsychotic induced catalepsy, which appears due to the blockade of dopamine neurotransmission\[^{[13]}\]. In the present study, ACP (10, 15 and 20% w/w) significantly (\(P<0.05, P<0.01\) and \(P<0.01\)) potentiated dose dependent haloperidol-induced catalepsy. Thus, the results suggest that ACP shows antidopaminergic activity.

Ketamine Induced stereotypy is a commonly employed interceptive behavioural model to evaluate antipsychotic potential of any drug. Haloperidol (antipsychotics agents) was used in the present study as standard antipsychotic agents. Administration of ACP in a specially prepared diet for 30 successive days in different concentrations showed significantly (\(P<0.05, P<0.01\)) inhibition of stereotypic behavior in mice as reflected by reduced turning, weaving, head-bobbing and locomotor activity. Administration of ACP for 30 successive days resulted in dose-dependent decrease in dopamine and serotonin level in brains of rats in the present study. A central role for D2 receptor occupancy in antipsychotic action is now well established, buttressed by neuroimaging studies using positron emission tomography and single photon emission computed tomography\[^{[14]}\]. However, the importance of dopamine receptors in the treatment of psychosis does not by itself constitute proof of the involvement of dopamine in psychosis. Administration of ACP may increase the number of dormant receptors, hence resulting in decrease in dopamine turnover in extracellular spaces in the pbrain\[^{[15]}\]. It derives that alkaloids, tannins, steroids and glycosides are present in the ACP which may possibly responsible for the psychopharmacological action. Pole-climb avoidance in rats is often used for differentiating neuroleptic activity and sedatives property. Administration of ACP for 30 successive days in different concentrations significantly (\(P<0.05, p<0.01\)) delayed the latency time taken by the animals to climb the pole in Passive Avoidance Paradigm. Since, ACP produced consistent antipsychotic activity in different antipsychotic models; it appears to be a promising antipsychotic agent.

**CONCLUSION**

The present investigation concludes that the *Allium cepa* paste contains constituents that inhibit dopaminergic neurotransmission and possibly blocks dopamine D2 receptor. Thus, ACP possesses antidopaminergic activity. The results suggest that the *Allium cepa* may have potential clinical application in the management of psychiatric disorders.
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