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An Investigation of Antipsychotic Activity of Plant Extracts in Experimental Animals

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Abstract

The current study is done on plant extracts as antipsychotic medication in ayurvedic formulation. The hallucinations are induced by electro shock therapy or apomorphine. After induction of hallucinations the plant extract is administered as standard group, control group and simple group of six albino rats and the results are screened and analyzed with standard drug comparision. Pre-treatment of MEIR (methanolic extract of *Ipomoea reniformis*) for 15days at the dose of 200 and 400 milligram per kilogram body weight was used to evaluate antipsychotic activity. The antipsychotic activity was also studied against Apomorphine induced stereotype behavior in rats, Pilocarpine induced purposeless chewing in rats and Apomorphine induced climbing behavior in mice. In pre-treatment studies of antipsychotic activity, MEIR at the dose of 200 and 400 milligram per kilogram body weight significantly ($p<0.001$) reduced the stereotyped behavior against Apomorphine induced stereotypy behavior model. In Pilocarpine induced purposeless chewing behavior, MEIR at the dose of 200 and 400 milligram per kilogram body weight significantly ($p<0.01$) decreased chewing behavior and in Apomorphine induced climbing behavior, MEIR significantly ($p<0.001$) reduced the climbing activity at the dose of 200 and 400 milligram per kilogram body weight. The results of the Pr (+) study suggest that the MEIR possess antipsychotic activity in rats and mice. The results of each activites are compared with standard medicament and evaluated.

Keywords: Ipomoea reniformis, apomorphine, locust bean gum, pilocarpine, hallucinations, antipychotic activity

1. INTRODUCTION

is psychosis or schizophrenia that is due to over release of dopamine neurotransmitter in limbic system. there is imbalance between dopamine and acetylcholine. This extra release of dopamine causes psychosis or uncontrolled speech, uncontrolled behaviour and uncontrolled function of body. The symptoms of psychosis can be treated with dopamine antagonists. Therers are some negative symptoms like blunted emotions, limited speech. There are two types of antichotic drugs (neuroleptics) typical or traditional or classic that control only positive sympotomes and atypical neuroleptics that control positive symptoms as well as negative symptoms of the patients.

The treatment of psychosis or schizophrenia is done with three major classes phenothiazine, thixanthines and butyrophenone. The popular medicaments are chlorpromazine, thioridazine, fluperzine, flupenthixol, haloperidole, droperidols etc. these medicines calm down the patient and compel to sleep and are known as major tranquilizers. These medicines also effect alpha 1adrenergic receptor and block this receptor that causes inhibition of ejaculation and loss of libido or impotency.

These also block cholinergic receptors and there are side effects of driness of mouth, atropy, sedation, drowsiness. The major side effects of these drugs are rigidity, tremor, akinesia, restlessness without anxiety, dardative dyskinesia, moon like face, linguafacial face muscle bizarre etc. These side effects are known as extra pyramidal side effects or parkinsonism-like symptoms that can be treated with anticholinergic medicines like trihexiphenydil. There is release of prolactine due to dopamine D2 receptor antiagonistic action. Dopamine inhibits release of prolactin.

These all dopamine D2 receptor antagonist blok the release of dopamine from granules of neutrons and controls positive symptoms while the atypical neutoleptics like clozapine, olanzapine and resperidone block dopamine D2 receptor and 5-HT2 antagonist that control positive and negative symptoms of psychosis patients. The drugs like reserpine mainly degrade granules in the neurons that release catecholamines like dopamine, noradrenaline and serotonin and is useful in treatement of psychosis due to inhibition of dopamine release from neutronal granules and hypertension due to inhibition of noradrenaline from neuronal granules and also causes suicidal attach as side effect due to

blockage of serotonin release from the neuronal granules.

2. MATERIALS AND METHODS

METHODOLOGY

Extraction of *Ipomoea reniformis*

This collected material was washed two times and then dried over night. This is then crushed and powdered and passed from sieve no 22 for unique particle size. This was then packed in column with n-hexane to remove fatty material and collected after 48 hours. Then it was packed in soxlet apparatus with methyl alcohol solvent to extract the active constituent from the material and the extract was washed to remove the methanol solvent and dried to make powder and for application. The clear solution was collected in siphon tube for further use[1].

Quality control and Screening of constituents

This process was performedagain and again till it gives fixed weight. Total ash % was determined by consideration with weight of initialpowder of plant material. Can be calculated as Total ash (% w/w) = (Wt. of ash/ sample Wt.) × 100 Acidinsoluble ash 2 gm of dried powder was added in pre weighed crucible of silica and burned at high temp lessthan 4500C until free from carbon. It was determined by cooling the silica dish in desiccator and weighted. The same process was repeated till constant weight was obtained. The ash obtained was mixed in 25 milliliter 2MHCl and boiled upto 7 min. Then not soluble content had been added in a silica crucibles. Again hot water addedand filtered, then burned and cooled in a desiccator, weight was taken.

. Water soluble ash Powder was taken and 2 gm powder was added in previously weighed crucible of silicaand it was then kept at high temperature not more than 4500C until it became free from carbon. It was determined by cooling the silica crucible in desiccator and weighted. The same process was repeated untilconstant weight was identified. The ash thus obtained was further boiled water was collected by filtration insilica plate and washed. The content was burned for few minutes at high temp. but not more than 4500C.

It was then heated at hightemperature not more than 4500C until it became free from carbon. It was determined by cooling the silicacrucible in desiccator and weighted. The same process was repeated till constant weight was identified. Theash obtained was mixed with 1 milliliter H2SO4, heated until release of white colored fumes finished, Furtherignited at 8000 ± 250C till all black particles get disappeared. The heating was done away from direct air[2].

The silica crucible was cooled. Again few drops of H2SO4 were added and ignited again. This process wasdone repeatedly to get constant weight. 2) Determination- Extractive values Water-soluble extractive valueMethod: 5 gm plant material powder was weighed was added in closed flask and kept for maceration in chloroform water (100 milliliter) then for 18 hours kept aside and filter. The percentage was determined byconsidering initial weight of plant material. Calculations: If twenty five milliliter aqueous filtrate produces X g of PPt, Then 100 milliliter of filtrate will give 4X g of residue, So 5 gm of powdered plant material contains 4X g of water soluble residue,So water soluble extractive value will be 80X. Alcohol-soluble extractive value: Method: Accurately weighed5 gm of powdered plant material was mixed with 95% ethanol (100 milliliter) in a closed vessel. It was maceratedfor 24 hours with occasional shaking for initial six hrs. Kept aside for 18 hours and filtered carefully to avoidevaporation of ethanol. Filtrate (25 milliliter) was evaporated in pre weighed porcelain dish, weight wascalculated. The percentage of alcohol soluble extractive value was determined by considering initial weightof powdered plant material stem. Calculations: 25 milliliter of alcohol filtrate possess about A g of residue, So 100milliliter of filtrate contains 4A gm of residue. Then this 100milliliter filtrate was prepared from 5 gm of powdered plantmaterial. So 5 gm of powdered plant material contains 4A gm of residue. And percentage of extractive valuewill be 80A gm of alcohol (90%) soluble residue Extractive Value (% w/w) = [(Wt. of residue ×100) / (25×sample wt.)] × 100 3) Determination of Crude fibre %w/w of crude fiber= (Wt. of dried residue - wt. ofincinerated residue)*100 Wt. of dried Sample 4) Determination of Volatile oil content Wt. of volatile oilcollected 5) Determination of swelling index According to the experiment protocol for each specific plant material, itis determined by adding water or a swelling agent (1 g) (whole, cut or pulverized). 6) Determination offoaming index Foaming index= = the volume of the decoction used for preparing the dilution Where foaming- 1 cm is observed. 7) LOD: The shallow glass-stopper weighing bottle was dried and weighed. 2g crudedrug was added in the bottle and closed, the weight was taken and crude drug was spread evenly to aheight not more than 10mm. Then the bottle was kept in the oven for drying keeping open without stopper. Again weighed loss on drying was calculated in percent w/w (Indian pharmacopoeia 1996). LOD=Loss in wt./sample wt. *100

Experiments for Evaluation of Antipsychotic Activity

1. Effect of *Ipomoea reniformis* on Apomorphine induced stereotype BEHAVIOR

Purpose and rationale

Apomorphine induces stereo typed behaving nature in rat, identified by gnawing, licking and sniffing in compulsive and respective way, that was an identification of striatial dopamine receptor excitation. medicaments that stop Apomorphine generated stereotype block and inhibit dopamine receptor into nigro striatal systems of brain.

Table 1:- Experimental design 4

Grp	Dose with treatment policy	Results
I	Apomorphine controlling (0.5% weight by volume SCMC l milliliter/100 g, peroral) + Apo-morphine (1.5 milligram per kilogram, intraperitonial)	Stereotypedbehavior
II	Halo-peridol (1 milligram per kilogram, intraperitonial) + Apo-morphine(1.5 milligram per kilogram, intraperitonial)	
III	*Ipomoea reniformis* (200 milligram per kilogram, peroral) + Apomorphine (1.5 milligram per kilogram, intraperitonial)	
IV	*Ipomoea reniformis* (400 milligram per kilogram, peroral)+ Apomorphine (1.5 milligram per kilogram, intraperitonial)	

Procedure

Rats had been alloted in 4 Grps further treatment were to following; Grp 1 found0.52 percent weight by volume SCMC l milliliter/100 gram, peroral further called as Apo-morphine controlling group, Grp 2 got HAL 1 milligram per kilogram, intraperitonial, Grp 3 along 4 obtained MEIR 200 and 400 milligram per kilogram, peroral respectivefully for 15 days. after half month, an hour after the administration of 0.5% weight by volume SCMC l milliliter/100 g, peroral in Grp I, 200 and 400milligram per kilogram, peroral MEIR in Grp III and IV respectively and 30min after the administration of Haloperidol (1milligram per kilogram, peroral) in Grp II, all Grps received Apo-morphine 1.5 milligram per kilogram, intraperitonial Stereo typed behavioring generated by Apo-morphine had been calculated each ten minutes upto ninety minutes immediate treatment of Apomorphine , that intervals are identified for study on the rats of behaviour. These rats had been personally kept into personal boxes along with observation of ten seconds. the results have been divided on the basis of the intensities of sleep cycle[3].

0 - no sleep or may come,1 - means Active for sleep,

2 - Sleep activity is predominant but alongwith bursting of stereo typed rearing and sniffing

3 – activity of locomotor is stul found with Continued stereo typing activities like, Head bobbing/rearing and sniifning

4 - Fixed stereo typing action maintaining to one position,

5 - Fixed stereo typed action but alongwith bursting of biling, gnawing/licking

6 - Continuous licking of boxes gridings along with,

7 - Continuous biting of boxes grids.

The technique tells about presence and absence of sniffing, billing, rearing in the continuous mode of administration of standard medicine and herbal dose of methyl alcoholic extract of Ipomoea reniformis.

Effect of *Ipomoea reniformis* on Pilocarpine induced purposeless chewingPurpose and rationale

The pharmacological activity of purpose less chewing or jaw locking is studing with interaction of neurotransmitter dopamine and acetylcholine. acetyl choline induced tremor and jaw locking movements are more prominent and powerful as compared to dopamine neurotransmitters and choniergics movements are studied in detail and antigoniszed by various anticholinergic and acetylcholine antagonistic medicaments as like pilocarpine, scopolamine and other effective medicines[4].

Table 2: Experimental design 5

Grp	Dose along with treatment	Results
I	Pilocarpine controlling (0.5% weight by volume SCMC l milliliter/100 g, peroral) + Pilocarpine (1 milligram per kilogram,intraperitonial)	Number of chewing's in 30min period.
II	Scopolamine (1 milligram per kilogram, intraperitonial) + Pilocarpine (1milligram per kilogram, intraperitonial)	
III	*Ipomoea reniformis* (200 milligram per kilogram, peroral) + Pilocarpine (1 milligram per kilogram, intraperitonial)	
IV	*Ipomoea reniformis* (400 milligram per kilogram, peroral) +Pilocarpine (1 milligram per kilogram, intraperitonial)	

Procedure

Rats had been distributed into 4 Grps and treatment were as following; Grp 1 got 0.51 percent weight by volume SCMC l milliliter/100 gram, peroral and knowing to Pilocarpine controlling group, Grp 2 obtained standard medicine Scopolamine 1milligram per kilogram, intraperitonial, Grp 3 and 4 got MEIR 200 along 400 milligram per kilogram, peroral respectively for 15days. On the 15 day, an hour after the administration of 0.5% weight by volume SCMC l milliliter/100 g, peroral in Grp I, 200 and 400milligram per kilogram, peroral MEIR in Grp III and IV regularly and 30 minute after the administration of Scopolamine (1milliliter/kg, intraperitonial) in Grp II, all Grps received Pilocarpine 1milligram per kilogram, intraperitonial After giving Pilocarpine the rats were placed individually in a large glass cylinder (height 30 cm, diameter 20 cm) at 23 ± 1°C and kept to habituating upto fifteen minutes before injection of medicaments. Numbers for chewing had been count by directly results soon after administration of medicament. The observations are Pr (+)ed as number of chewing for thirty min time[5].

Effect of *Ipomoea reniformis* on climbing behavior generated by apomorphine

Purpose and rationale

Neuroleptic activity is correlated with apomorphine generated climbing behaviors of rats. The apomorphine medicament administered to rats that produce climing behavior initially and after some time actively showing climbing nature. The behavior is changed to dopamine releasing activity in misolimbic region and the rat try to climb in cage or water tub. The effect is studied by antagonistic action to the rats.

Table 3:- Experimental design 6

Grp(n=6)	Dose along with treatment	Result
I	Apomorphine controlling(0.5% weight by volume SCMC l milliliter/100 g, peroral)+ Apomorphine (3 milligram per kilogram, intraperitonial)	Climbingbehavior
II	Haloperidol (0.1 milligram per kilogram, intraperitonial) + Apomorphine (3 milligram per kilogram, intraperitonial)	
III	*Ipomoea reniformis* (200 milligram per kilogram, peroral) + Apomorphine (3 milligram per kilogram, intraperitonial)	
IV	*Ipomoea reniformis* (400 milligram per kilogram, peroral) + Apomorphine (3 milligram per kilogram, intraperitonial)	

Procedure

Mice had been categorized into four Grps and treatment had beeb as following; Grp 1 obtained 0.53 percent weight by volume SCMC l milliliter/100 gram, peroral then called as Apomorphine controlling group, Grp 2 obtained standard medicament Haloperidol 0.1 milligram per kilogram, intraperitonial, Grp 3 and 4 obtained MEIR 200 and 400 milligram per kilogram, peroral contineously for 15 days. after half month, an hour after the administration of 0.55 percent weight by volume SCMC l milliliter/100 gram, peroral in Grp 1, 200 alongwith 400 milligram per kilogram, peroral MEIR in Grp 3 alongwith 4 respectively and 30min after the administration of standard Haloperidol (0.1milligram per kilogram, intraperitonial) in Grp II, all Grps received Apomorphine 3 milligram per kilogram, intraperitonial after giving Apomorphine, the mice had been kept separatelu in wiring mesh wood boxes. Then they were observed for climbing behavior for each 10 minutes for 30 minutes soon after Apo-morphine cure and scored[6].

0 = 4 paws on the floor,

1 = fore feet holding the vertical bars, 2 = 4 feet holding the bars.

STATISTIC RESULTS

These results had been calculated as mean ± mean ofStandard error. Statistics comparison had been carried out by one way Analysis of variance further proceeded by Tukey post experiment utilizing Graphic Pading Prism version 9.0, United States America. P< 0.05 has been known important.

3. RESULTS AND DISCUSSION

The antipsychotic activities are produced by similar procedure. The four Grps of animals were made for experimental study. Each Grp contains 6 animals like rats, mice, rabbit for experiment. The animals were treated with methyl alcohol extract of plant Ipomoea reniformis. The control Grp was administered with agent that can induce psychosis in animals and standard antipsychotic medicament was given to standard Grp. The readings were obtained and analyzed with stastics like ANOVA, t-experiment, F-experiment, standard deviation, standard error, average mean of all six reading. The one way anova is generally utilized in calculation of readings and data obtained during experimental study.

Table 4: Effect of MEIR on Apomorphine induced stereotypi in rats.

Grps	Treat-ment	Stereotype Scores at			
		10 min	**20 min**	**30 min**	**40 min**
I	Apomorphine Control	3.18 ± 0.65	3.85 ± 0.19	4.18 ± 0.17	4.15 ± 0.14
II	Haloperidol (1 mg / kg)ip	2.58 ± 0.61	1.68 ± 0.24	0.17 ± 0.15	0
III	MEIR (200 mg)peroral	3.15 ± 0.19	367 ± 34	3.68 ± 0.44	3.16 ± 0.32
IV	MEIR (400 mg) peroral	3.63 ± 0.34	3.52 ± 0.43	4.18 ± 0.48	3.64 ± 0.30

Table 5: Effect of MEIR on Apomorphine induced stereotypi in rats.

Grps	Treat-ment	Stereotype Scores at				
		50 min	**60 min**	**70 min**	**80 min**	**90 min**
I	Apomorphine Control	4.17 ± 0.16	4.35 ± 0.19	4.65 ± 0.19	4.85 ± 0.14	4.02 ± 0.87
II	Haloperidol (1 mg/kg)ip	0	0	0	0	0
III	MEIR (200 mg)peroral	3.65 ± 0.20	367 ± 34	2.68 ± 0.44	2.35 ± 0.19	1.66 ± 0.32
IV	MEIR (400 mg) peroral	3.83 ± 0.34	2.82 ± 0.43	2.48 ± 0.48	1.65 ± 0.19	1.64 ± 0.30

Values have been given in mean ± standard error mean (n=6)

Grp I = Apomorphine control (0.5% weight/vol sodium carboxymethyl cellulose 1 milliliter per 100 gram peroral + Apomorphine)

Grp II = Haloperidol (5 milligram per kilogram intraperitonial + Apomorphine)

Grp III = Methanolic extract of Ipomoea reniformis (200 milligram per kilogram by orally + Apomorphine)

Grp IV = Methanolic extract of Ipomoea reniformis (400 milligram per kilogram by orally + Apomorphine

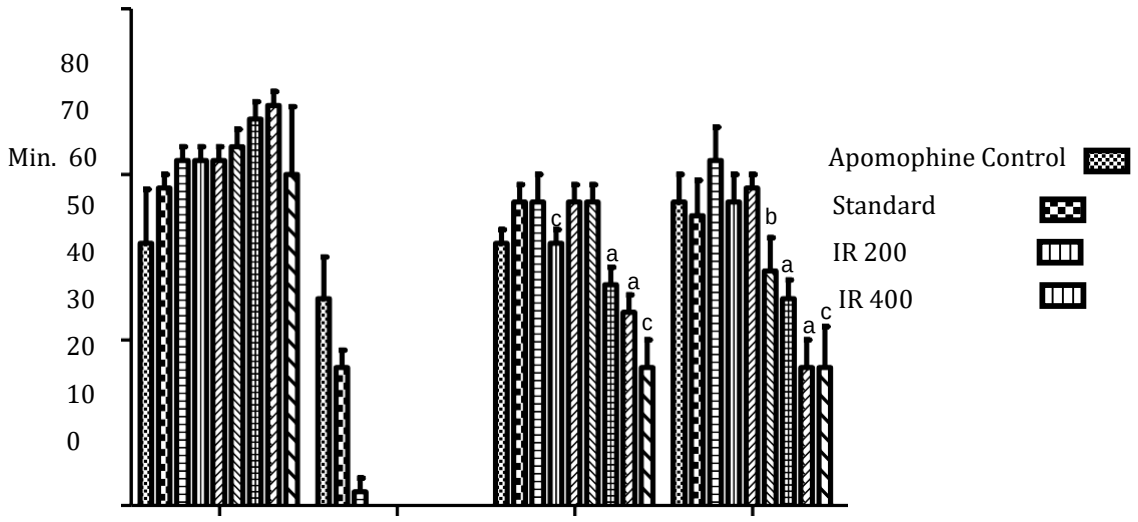

Figure 1: Effect of MEIR on Apomorphine induced stereotypy in rat

Values are expressed in mean ± standard error mean; rePr (+)s different phases of convulsions in seconds (where n=6).

*Haloperidol had complete blocked the hind limb or leg extensor phase. A = $p < 0.001$, b = $p < 0.01$, c = $p < 0.05$; compared with Pentylene tetrazole PTZ control Grp.

Statistical analysis has done by one way A.N.O.V.A. followed by Tukey's experiment.

Table 6: Effect of MEIR on Pilocarpine induced purposeless chewings**.**

Grps	Treatment	Number of Chewing in 30 minutes
I	Pilocarpine Control	782.13 ± 12.94
II	Scopolamine (1 milligram per kilogram intraperitonial)	519.05 ±48.24
III	MEIR (200 mg)peroral	688.30 ±36.07
IV	MEIR (400 mg) peroral	555.06 ± 53.83

Values have been given in mean ± standard error mean (n=6)

Grp I = Pilocarpine control (0.5% weight/vol sodium carboxymethyl cellulose 1 milliliter per 100 gram peroral + Pilocarpine)

Grp II = Scopolamine (1 milligram per kilogram orally + Pilocarpine)

Grp III = Methanolic extract of Ipomoea reniformis (200 milligram per kilogram by orally + Pilocarpine)

Grp IV = Methanolic extract of Ipomoea reniformis (400 milligram per kilogram by orally + Pilocarpine)

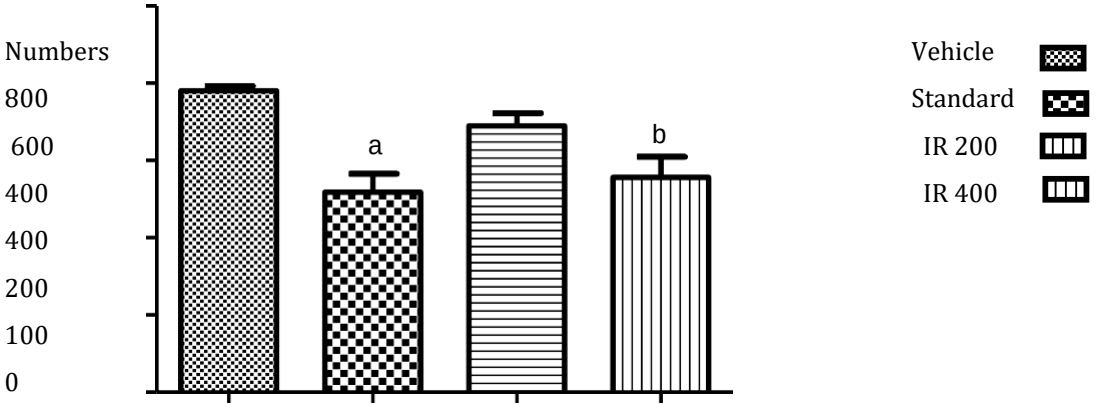

Figure 2: Effect of MEIR on Pilocarpine induced purposeless chewings

Values are expressed in mean ± standard error mean; rePr (+)s number of purposeless chewing in 30 minutes (where n=6).

*Scopolamine had complete blocked the hind limb or leg extensor phase. A = $p < 0.001$, b = $p < 0.01$, c = $p < 0.05$; compared with M.E.S. control Grp.

Statistical analysis has done by one way A.N.O.V.A. followed by Tukey's experiment.

Table 7: Effect of MEIR on Apomorphine induced climbing behavior in Mice.

G	Treatment	Climbing Behavior Score		
		10 min	**20 min**	**30 min**
I	Apomorphine Control	1.50 ± 0.21	1.66 ± 0.22	1.64 ± 0.23
II	Haloperidol (1 milligram per kilogram intraperitonial)	0	0	0
III	MEIR (200 mg)peroral	0.65 ±0.42	0.51 ±0.31	0.32 ±0.22
IV	MEIR (400 mg) peroral	0.66 ± 0.43	0.34 ± 0.31	0.16 ± 0.17

Values have been given in mean ± standard error mean (n=6)

Grp I = Apomorphine control (0.5% weight/vol sodium carboxymethyl cellulose 1 milliliter per 100 gram peroral + Apomorphine)

Grp II = Haloperidol (0.1 milligram per kilogram orally + Apomorphine)

Grp III = Methanolic extract of Ipomoea reniformis (200 milligram per kilogram by orally + Apomorphine)

Grp IV = Methanolic extract of Ipomoea reniformis (400 milligram per kilogram by orally + Apomorphine)

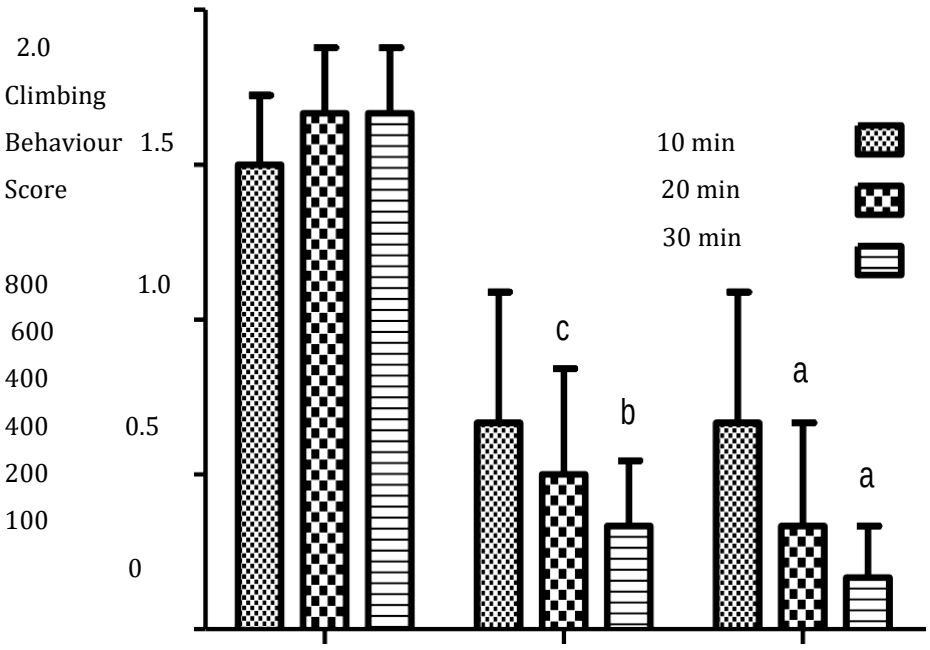

Figure 3: Effect of MEIR on Apomorphine induced climbing behavior in mice

Values are expressed in mean ± standard error mean; rePr (+)s number of purposeless chewing in 30 minutes (where n=6).

*Haloperidol had complete blocked the hind limb or leg extensor phase. A = $p < 0.001$, b = $p < 0.01$, c = $p < 0.05$; compared with M.E.S. control Grp.

Statistical analysis has done by one way A.N.O.V.A. followed by Tukey's experiment.

DISCUSSION

The results of the Pr (+) study on animals indicate that *Ipomoea reniformis* possesses anti-psychotic activity against stereotype behavior induced by Apomorphine, Pilocarpine induced purposeless chewing and against climbing behavior induced by Apomorphine.

Apomorphine induces stereotyped behavior in rats via stimulation of the central dopaminergic receptors. The ability of a drug to antagonize Apomorphine-induced stereotyped behavior in the rodents has been correlated with neuroleptic activity. Inhibition of Apomorphine-induced stereotyped behavior in the rodents is suggestive of D_2 receptor blockade. [65] The ability of the *Ipomoea reniformis* to antagonize Apomorphine-induced stereotyped behavior in the rodents supports the hypotheses of central activity of *Ipomoea reniformis* which might be related to anti-dopaminergic, anti-serotonergic and GABA mimetic actions.

The pharmacology of vacuous jaw movements (purposeless chewing) is characterized by an acetylcholine/dopamine interaction. Cholinergic stimulation in rats appears to be more effective than interfering with DA systems as a method for inducing vacuous jaw movements. Cholinomimetic-induced vacuous jaw movements can be reduced by the DA agonist Apomorphine.

SUMMARY AND CONCLUSION

In pre-treatment (15days) study of antipsychotic activity, *Ipomoea reniformis* significantly ($p<0.001$) reduced the stereotyped behavior against Apomorphine induced stereotypy behavior model. In Pilocarpine induced purposeless chewing model, *Ipomoea reniformis* significantly ($p<0.001$) decreased the chewing behavior and in Apomorphine induced climbing behavior, *Ipomoea reniformis* significantly ($p<0.01$) reduced the climbing activity

We also conclude that *Ipomoea reniformis* possess anti-psychotic activity against stereotype behavior induced by Apomorphine, Pilocarpine induced purposeless chewing and against climbing behavior induced by Apomorphine.

The anti-psychotic activity of *Ipomoea reniformis* was evaluated in Apomorphine induced stereotype behavior, Pilocarpine induced purposeless chewing in rats and Apomorphine induced climbing behavior in mice.

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