Original Article

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Evaluation of Antipsychotic Activity of Ethanolic Bark Extract of *Myrica esculenta* in Rats

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ABSTRACT

The antipsychotic properties of *Myrica esculenta* stem bark were evaluated. The stem bark was collected, shade dried, and pulverized. Extraction was carried out with 70% ethanol by occasional shaking. Preliminary phytochemical screening of the extract was investigated in this study. Antipsychotic activity was evaluated against apomorphine-induced stereotypy using cook's pole climbing apparatus and haloperidol-induced catalepsy models. Bioamine determination of noradrenaline and dopamine was also performed. The extract contains phytochemicals, including glycosides, flavonoids, volatile oils, proteins, saponins, phenolics, and tannins. The result showed decreased apomorphine-induced stereotyped behavior. This study reported significant dose-dependent potentiation of haloperidol-induced catalepsy in rats and a longer time needed by the rat to climb the pole in a dose-dependent manner. Also, it significantly decreased brain dopamine and noradrenaline level. The ethanolic extract of *M. esculenta* exhibited significant antipsychotic activity in rats. Further neurochemical investigation is needed to explore the plant drug's mechanism of action regarding anti-dopaminergic functions and establish the plant as an antipsychotic agent.

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INTRODUCTION

The traditional system of medication has given knowledge for the discovery of new valuable drugs. The modern and advent techniques of drug screening and drug development of traditional medicine have values and importance in many people. In the world, 80% of the population still use traditional medicine as their primary source of medication (Surveswaran et al., 2007). Due to the modern isolation techniques and pharmacological testing procedures, new plant drugs get their way to the modern medication system (Jain, Jain, and Shete, 2010). *Myrica esculenta* Buch – Ham. (Family: Myricaceae) is a traditional plant used in ayurvedic medicine for its catarrhal fever, cough, throat infection, asthma, urinary discharges, bronchitis, psychiatric disorder anemia, cholera, ulcers, and in treatments of various disease (Bhatt, Rawal, and Dhar, 2000). In the present study, an attempt has been made to explore the psychoactive potential of the ethanolic extract of *M. esculenta* in apomorphine-induced stereotyped behavior, cook's pole climbing apparatus, and haloperidol-induced catalepsy models. Bioamine determination of noradrenaline and dopamine was also performed.

Myrica esculenta Buch – Ham. (Fam., Myricaceae) Syn., *M. nagi* Hook. F., commonly known as Kaiphala or Katphala is an evergreen dioecious tree distributed in the subtropical Himalayas. It is distributed in India, Nepal, China, Pakistan, and Malaysia Islands. The plant is commonly found in the outer Himalayan region at an altitude starting from about 900 m up to 2,100 m. It is a medium to a large woody, evergreen, dioecious tree, attains a height of 12 to 15 meters (Sood and Shri, 2018). Leaves are lanceolate, 9 cm long, 3 cm broad, lower surface-pale green, upper surface-dark green. Generally, leaves are crowded towards the end of branches. The bark is grey to brownish-grey, inner surface dark brown in color and smooth, fracture hard, and tastes bitter. Flowers are minute, unisexual and glandular, sessile, solitary, and bracteate; sepals and petals, either absent or not visible; inflorescence, a catkin, 4.2 cm long, axillary, bearing about 25 flowers; only a thread-like style visible with the unaided eye. Fruits are drupe, ellipsoid, or ovoid-shaped, in length 0.7-1.0 cm, 0.5-0.7 cm wide dark brown colored. Seeds are ovoid 0.6 cm long and 0.3 cm wide. The seed's surface is very smooth, light brown in color, and oily in the test. The plant prefers light (sandy) and medium (loamy) soils and requires well-drained soil with 250 - 205 cm rainfall (Kabra et al., 2019a; Sood and Shri, 2018). A variety of constituents has been isolated from *M. esculenta*, and their structures were elucidated. They belong to different classes such as glycosides, flavonoids, volatile oils, proteins, saponins, phenolics, and tannins (Srivastava et al., 2016).

METHODS

Collection of Plant Material

The stem bark of *M. esculenta* was collected from the western hills of Nepal. The plant was identified and authenticated by Dr. N.M. Ganesh Babu, a botanist at FRLHT (Foundation for Revitalisation of Local Health Traditions) Jarakabande Kaval, post Attur, Yehalanka, Bangaluru (560106). A herbarium specimen of the plant was prepared and kept at the pharmacognosy laboratory of college for future references.

Extraction

Dried and powdered stem bark (100 g) was extracted with 70% ethanol with occasional shaking for 24 hours.

Phytochemical Analysis

Phytochemical analysis was carried out to test its chemical constituents. All the chemicals used for testing were of analytical grade, and procedures were based on the standards protocols (Sapkota and Jain, 2020).

Animals

Male albino rats (150-200 g) were used in this study. All the rats were placed in an animal house with room temperature (24 °C ± 2) and humidity (60-70%). The animal house was cleaned occasionally. Food with ad libitum of water was supplied to all animals. The experimental procedure was performed as per the international ethics committee guideline only after the approval from the Institutional Animal Ethics Committee (IAEC) (Sahoo et al., 2016).

Pharmacological Studies

1. Apomorphine-induced stereotypy in rats (Erbaş et al., 2013)
2. Haloperidol-iduced catalepsy (Nishchal et al., 2014)
3. Pole climb avoidance in rats (Madhav, 2015)

Bioamine Estimation in Rat Brain

Collection of brain sample

Rats were sacrificed by decapitation process. The brain was collected rapidly, washed properly, made free from blood, and stored at –20 °C. Thus, the weight of the brain was measured for amine determination. After thawing, homogenization of the brain was done with ice-cold 0.01 N HCl and 0.1 ml 10% EDTA. Homogenate was mixed properly by shaking with 25 ml n-butanol and 4 g NaCl. Finally, the mixture was centrifuged and kept at room temperature for 20 min. Then, 24 ml n-butanol, 40 ml n-heptane, and 1.5 ml 0.5 M phosphate buffer (*p*H 7.3) were added. The mixture was shaken for 10 min and settled for 10 min. After that, 1.5 ml phosphate buffer layer was taken. It was acidified to pH 3.5 to 4.0 with 3N HCl. Peroxide free ether (20 ml) was added to it and shaken for 10 min. Aqueous acid layer (0.5 ml) was taken for fluorometric estimation of noradrenaline and dopamine, etc., following Welch and Welch's method.

1. Estimation of noradrenaline (Brownlee and Spriggs, 1965; Welch and Welch 1969)
2. Estimation of dopamine (Brownlee and Spriggs, 1965; Welch and Welch, 1969)

Statistical Analysis

Results were expressed as mean ± SEM, (n=6). Statistical analyses were performed with one -way analysis of variance (ANOVA) followed by Tukey Multiple Comparison Test by using Graph Pad Prism. A P-value less than 0.05 was considered to be statistically significant.

RESULTS

Phytochemical Investigation in The Bark of *M. esculenta*

Phytochemical screening was performed in this study. It showed various phytochemicals like carbohydrates, saponins, flavonoids, phenolics, glycosides, proteins, volatile oils, tannins, and mucilage. It also indicated that alkaloids, fat and fatty acids, phytosterols, and flavone glycosides were absent. The details of the phytochemical screening are given in Table 1.

Table 1: Phytoconstituents present in the bark extract of *M. esculenta*

No	Phytoconstituents	Result
1	Carbohydrates	+
2	Alkaloids	-
3	Saponins	+
4	Flavonoids	+
5	Phenolics	+
6	Fat and Fatty acids	-
7	Glycosides	+
8	Proteins	+
9	Volatile oils	+
10	Tannins	+
11	Phytosterols	-
12	Mucilage	+
13	Flavone glycosides	-

(+) indicates presence and (-) indicates absence of Phytochemicals

Table 2: Effect of ethanolic extract of *M. esculenta* on apomorphine-induced stereotyped behavior

Treatment	Stereotype score (mean ± S.E.M.) at								
	10 min	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**	**70 min**	**80 min**	**90 min**
Normal	3.34±0.32	4.68± 0.23	4.73±0.27	5.16±0.41	4.61±0.71	4.21±0.67	2.79±0.61	3.11±0.58	2.41±0.31
HAL	0.61±0.2^{9}*	1.12±0.41***	1.42±0.19***	0.86±0.54***	0.32±0.22***	0.69±0.31**	1.26±0.19*	1.67±0.38**	0.32±0.22*
ME 200	2.21±0.48	2.85±0.40**	2.65±0.61**	2.49±0.56**	1.47±0.22**	1.84±0.40*	2.35±0.76	2.05±0.41	2.72±0.58
ME 400	1.94±0.30	2.67±0.21***	2.30±0.42***	1.17±0.30***	1.02±0.26***	1.34±0.42*	0.69±0.21	0.79±0.31**	1.32±0.49

(Values are expressed in mean ± S.E.M., where HAL= Haloperidol, ME= Myrica esculenta, n = 6. * P<0.05 ** P<0.01 ***P<0.001; compared with vehicle treated group)

Table 3: Effect of ethanolic extract of *M. esculenta* on haloperidol-induced catalepsy

Treatment	Catalepsy score (mean ± S.E.M.) at					
	15 min	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Normal	0±0	0±0	0±0	0±0	0±0	0±0
HAL	54.25±1.95	52.95±1.60	54.83±2.13	53.93±2.53	56.45±1.08	58.12±0.57
ME 200 + HAL	55.48±1.08	54.62±0.95	57.22±1.73	58.60±1.74	58.18±1.18	58.90±1.11
ME 400 + HAL	59.28±1.53	59.07±0.67**	64.02±2.01*	62.75±1.37*	61.38±1.51*	61.75±1.97

(Values are expressed in mean ± S.E.M., where HAL= Haloperidol, ME= Myrica esculenta, n=6. *P<0.05; compared with vehicle treated group)

Table 4: Effect of ethanolic extract of *M. esculenta* on condition avoidance response

No.	Treatment	No. of Time Escaped	% Reduction in No. Of Times Escaped
1.	Control	16 ± 1.97	0
2.	HAL	9 ± 1.23	43.75
3.	ME (200 mg/kg)	12 ± 2.64	25
4.	ME (400 mg/kg)	11 ± 2.97	31.25

(Values are expressed in mean ± S.E.M, where ME= Myrica esculenta, HAL= Haloperidol)

Apomorphine-Induced Stereotyped Behaviors

Animals pre-treated with of 200 mg/kg dose of extract produced significant (P<0.05 and P<0.01) reduction in stereotyped score at 20, 30, 40, 50. and 60 min time interval as compared to the vehicle-treated animals. A higher dose of *M. esculenta* (400 mg/kg) produced a significant (P<0.001) reduction in the stereotyped score at 10–50 min time intervals. The effect is shown in Table 2.

Haloperidol-Induced Catalepsy

M. esculenta extract-treated rat did not show different effects and appeared the same as the vehicle-treated animals, so it did not induce catalepsy in rat. The cataleptic effect produced by haloperidol (1 mg/kg *ip*) was not affected by extract of 200 mg/kg dose while 400 mg/kg produce significant effect and (P<0.05 and P<0.01) potentiate the cataleptic effect of haloperidol at 15, 30, 45, 60, 75 and 90 min time intervals. The effects are shown in Table 3.

Pole Climbing Avoidance in Rats

All the groups (i.e. HAL (1 mg/kg) = 9±1.23, *M. esculenta* (200 mg/kg) = 12±2.64 and *M. esculenta* (400 mg/kg) = 11±2.97) significantly (P<0.05) decreased the escape response compared to the vehicle treated group (i.e. 16±1.97). Haloperidol (1 mg/kg) reduced the escape response by almost 43%, *M. esculenta* (200 mg/kg) by 25% and *M. esculenta* (400 mg/kg) by 25%. It is well depicted in Table 4.

Bio Amine Estimation

Nor adrenaline

The present spectrophotometric analysis showed a decrease in the NA level in all test groups compared to the control group. *M. esculenta* 200 mg/kg, 400 mg/ kg, and haloperidol pretreatment in rats exhibited a significant reduction in brain noradrenaline level compared to control group. The details are shown in Table 5.

Dopamine

The present spectrophotometric analysis showed a reduction in the dopamine level in all test groups as compared to the control group. *M. esculenta* 200 mg/kg, 400 mg/ kg, and haloperidol pretreat *M. esculenta* in rats exhibited significant reduction in brain dopamine level (P<0.05, P<0.001 and P< 0.001, respectively) compared to control group.

Table 5: Noradrenaline (ng/mg) level in rat brain

No	Group	Noradrenaline in (ng/mg) Mean ± SEM
1.	Control	589.70 ± 28.44
2.	HAL	541.40 ± 30.54***
3.	ME (200 mg/kg)	579.14 ± 28.26*
4.	ME (400 mg/kg)	563.23 ± 27.13***

*(Values are expressed in mean ± S.E.M., *P<0.05 **P<0.01 ***P<0.001; compared with vehicle treated group)*

Table 6: Dopamine level (ng/mg) in rat brain

No	Group	Dopamine in (ng/mg) Mean ± SEM
1.	Control	2364 ± 43.34
2.	HAL	2015 ± 146.40***
3.	ME (200 mg/kg)	2296 ± 186.30*
4.	ME (400 mg/kg)	2065 ± 170.10***

*(Values are expressed in mean ± S.E.M., where *P<0.05 **P<0.01 ***P<0.001; compared with vehicle treated group)*

DISCUSSION

In the present study, the antipsychotic effects of the hydroalcoholic extract of *M. esculenta* bark were studied in several behavioral animal models to evaluate their possible psychotropic activity. The present investigation results showed that the ethanolic extract of *M. esculenta* bark has some potent antipsychotic activity.

Firstly, the extract was tested in apomorphine-induced stereotyped behavior in rats, which is the classical model for antipsychotic effects (Protais, Costentin, and Schwartz, 1976). In this model, apomorphine, a non-selective dopamine agonist, induces stereotyped behavior such as locomotor hyperactivity, climbing, stereotyped grooming, licking, and gnawing. The ability

of test agents to inhibit these behaviors is a measure of its antipsychotic effect (Protais, Costentin, and Schwartz, 1976). This model is largely based on the dopamine theory of schizophrenia. In this study, as expected, apomorphine-induced stereotypy behavior was inhibited by both the extract and the reference drug, haloperidol. Experimental studies have shown that phytochemicals, particularly flavonoids and vitamins, present in *M. esculenta* are important antioxidants and superoxide scavengers. The antioxidant activity of *M. esculenta* may be responsible for its beneficial antipsychotic action (Srivastava et al., 2016).

Accordingly, the extract was tested in a haloperidol-induced cataleptic model in rats. In this model, haloperidol, a typical neuroleptic agent, induced a cataleptic state in rodents, which tested the extrapyramidal side effects of antipsychotic agents. Haloperidol is a well-known neuroleptic, primarily acting as a D2 receptor antagonist in the mesolimbic and mesocortical pathways. Due to its non-selective action, it also produces blockade of postsynaptic D2 receptors in the nigrostriatal pathway leading to the development of extrapyramidal side effects in humans and catalepsy in animals (Sanberg, 1980). Several other neurotransmitters such as acetylcholine, serotonin, angiotensin, adenosine, or opioids have also been implicated in the catalepsy induced by neuroleptic agents (Polydoro et al., 2004). Along with neurotransmitters in catalepsy, reactive oxygen species have also been proposed to play a role in haloperidol-induced toxicity (Polydoro et al., 2004). Several earlier behavioral studies have demonstrated dopamine facilitator activity and the antioxidant properties of *M. esculenta*, and it has been claimed to give remarkable protection against lipid peroxidation (Kabra, et al., 2019b; Kabra, et al., 2019c). Since reactive oxygen species have been implicated in haloperidol-induced toxicity, it can be safely assumed that the antioxidant property of *M. esculenta* may contribute towards its anticataleptic activity too.

Similarly, pole-climb avoidance is often used for differentiating neuroleptic activity and sedatives property in rats. Administration of *M. esculenta* 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 the Passive Avoidance Paradigm.

CONCLUSION

The present study demonstrates that *M. esculenta* has a protective effect against apomorphine-induced stereotypy, haloperidol-induced catalepsy, and pole climb avoidance test comparable to the standard drug trihexyphenidyl. Our study indicates that *M. esculenta* could be used as an alternative/adjuvant drug in preventing and treating symptoms of psychotic conditions. However, it requires further preclinical and clinical studies to prove it.

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