RADICAL SCAVENGING AND ANTISTRESS ACTIVITY(S) OF *MUSSAENDA FRONDOSA* ROOTS (RUBIACEAE)

Sameksha Koul*, Anu Chaudhary

Department of Pharmacy Bharat Institute of Technology, Partapur, By-Pass road, Meerut-250103, India

***Corresponding Author:** Sameksha Koul, School of Pharmacy, Bharat Institute of Technology, Meerut-250103, Email: samekshakoul@gmail.com Tel: +91-121-2440480

Summary

The plant Mussaenda frondosa (Rubiaceae) is known as white lady in English and bedina in hindi. Earlier claims show that the plant contains hypercin, quercetin, β-sitosterol glucoside. Ethanolic and aqueous extracts of the plant showed the presence of flavanoids, phenolic compound, tannins and anthocyanins. The present study was designed to investigate the in vitro free radical scavenging effect and stress induced changes in brain neurotransmitters as well as monoamine oxidase levels in albino rats in different models. The ethanolic extract of Mussaenda frondosa roots extract was found to possess normalizing activity against cold immobilization stress induced changes in norepinephrine (NE), dopamine (DA), 5-hydroxy tryptamine (5-HT), 5-hydroxy indole acetic acid (5-HIAA), and enzyme monoamine oxidase (MAO). The results obtained provide biochemical evidence for antistress activity of the tested extract. The inhibitory concentrations (IC_{50}) in DPPH, Superoxide scavenging activity and hydroxide scavenging activity were found to be 51.3, 24.6 and 52.7 µg/ml respectively. The above results suggest that ethanolic root extract of Mussaenda frondosa exhibits potential free radical scavenging effect that can reduce the oxidative stress. Thus the study provides the free radical scavenging and antistress actions of the extract.

Keywords: *Mussanda frondosa,* Radical scavenging activity, Antistress.

Introduction

Stress is a daily phenomenon faced by every human, normal functioning of every individual is dependent on optimum levels of stress. It is a vital that stress is kept under control and normal functioning is not hampered due to excess of stress [1]. Free radical induced per-oxidation has gained much importance because of their involvement in several pathological conditions such as cancer, cardiovascular disease, liver, diabetes mellitus, inflammation, renal failure, brain dysfunction and stress among others. Healthy cells can scavenge free radicals effectively by means of antioxidants. Antioxidant can act by scavenging reactive oxygen species by inhibiting their formation, by binding transition metal ions and preventing the formation of hydroxyl and/ or decomposing of lipid peroxides, by repair damage or by combination of all [2]. Many marketed formulations claim to possess antioxidant and anti stress actions, but still many herbs which have claims to be general tonics need to be investigated and their claims be authenticated. In recent era there is great thrust on screening of herbs for such activities. With this observation the present study was designed to investigate the antioxidant and antistress activity of Mussaenda frondosa root extract.

*Mussaenda frondosa syn Mussaenda glabrata (Rubiaceae) found in t*ropical Himalayas, Khasi Hills, Deccan Peninsula and the Andamans. Different parts of this plant are used as (Flower) diuretic, antiasthmatic, antiperiodic. Leaves and flowers—used in external applications for ulcers. Root— used in the treatment of white leprosy. White petiolate bract—prescribed in jaundice. The flowers contain anthocyanins, hyperin, quercetin, rutin, ferulic and sinapic acids; beta-sitosterol glucoside. *Mussaenda glabra* Vahl (tropical Himalayas from Nepal eastwards, Bihar, Bengal and Assam) is known as Sonaaruupaa in Assam. An infusion of the leaves is used for cough, asthma, recurrent fevers; also as a diuretic in dropsy [3].

Material and Methods

Plant material:

The *Mussaenda frondosa* root was procured from the Plant Physiology Division, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Krishi Nagar, Jabalpur, (M.P.) and authenticated by Dr. Anjula Pandey, taxonomic division, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic and Resources, Pusa Campus, New Delhi. Voucher specimens NHCP/NBPGR/2009/98/2225 dated 22/08/2009. The voucher specimen was retained in our laboratory for future reference.

Preparation of plant extract:
The plant material was dried under shade, reduced to moderately coarse powder and was extracted successively with ethanol using soxhlet apparatus. The ethanolic extract was dried under vacuum (yield 10.78%) and then suspended in 20% (v/v) propylene glycol –water to give a concentration of 500 mg/ml.

Animals:
The Institutional Animal Ethics Committee, (IAEC) review the protocol and approved the use of animals for the studies, (Ethical clearance number: 1147/ab/07/CPCSEA). Wistar albino rats of both sexes (150±20g b.w.) were used for the present studies. They were housed in clean polypropylene cages (3 in each cage) and maintained under standard laboratory condition at an ambient temperature 25±2°C with 55-64% relative humidity and 12 h light – dark cycle. They were allowed free access to standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libidum*.

In-vitro antioxidant activity:

Determination of the scavenging activity of superoxide radical:

The above activity was determined by the nitroblue tetrazolium (NBT) reduction method. The percentage inhibition of super oxide generation was evaluated by comparing the absorbance values of the control and experimental tubes. Vitamin C used as standard. [4].

Determination of the scavenging activity of hyrodxyl radical

The scavenging capacity for hyrodxyl radical was measured according to the modified method of Halliwell. The hydroxyl radical scavenging activity of the extract is reported as percentage inhibition of deoxyribose degradation. Vitamin C used as standard. [5].

DPPH radical scavenging activity

The free radical scavenging activity of the extract and butylated hydroxyl toluene (BHT) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. Vitamin C and BHT were used as standard. [6].

Nitric oxide scavenging

Nitric oxide was generated from sodium nitroprusside and neasured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated by use of Griess reagent. The absorbance of the chromophore formed during the deionization of nitrite with sulphanilamide and subsequent coupling with napthlyethlenediamine was read at 546nm and reffered to the absorbance of standard solutions of potassium nitrite, treated in the same way with griess reagent. Vitamin C and Curcumin were used as standard. [7].

Assessment of activity:

The rats were divided in to four groups of six rats in each. Group I served as control, Group II served as restraint control and Group III Mussaenda frondosa treated. The control animals received vehicle (1ml), and the treated group received the extract as a suspension at a dose of 500 mg/kg, b.w. once daily in the morning for 16 days through gastric intubation. One hour after the administration of the last dose, stress was induced by individually placing the animals in a restrainer for 3 h at 4°C [8]. Thereafter, the animals were sacrificed by cervical dislocation , whole brain was rapidly frozen at -5°C and brain NE, DA, 5-HT, 5-HIAA were spectrofluorimetrically estimated by the methods of Ansell and Beeson [9] as modified by Cox and Perhach [10]. Brain MAO levels was estimated spectrometrically by McEween's method.

Statistical analysis

All observations are presented as mean ± SEM. The data was analyzed by student's-*t* test. Differences were considered significant at the 5% level.

Result and discussion

Several concentrations ranging from 2 to 500 µg/ml of the ehtanolic extract of Mussaenda frondosa were tested for their antioxidant activity in different in vitro models. It was observed that free radicals were scavenged by the test compounds in a concentration-dependent manner up to the given concentration in all the models.

The maximum inhibitory concentrations (IC_{50}) in all models, viz. DPPH, Superoxide scavenging activity, hydroxide scavenging and nitric oxide activity were found to be 51.3, 24.6 and 52.7 and 52.3 µg/ml, respectively (Table-1)

Drug treatment was found to prevent the stress induced depletion of norepinephrine and dopamine levels thus helping the organism to cope up better during stress. Pretreatment with plant extracts was found to significantly reduce the stress induced rise in brain 5-HT, 5-HIAA levels by preventing the alarm reaction which elicits a significant rise in 5-HT and 5-HIAA levels [10], thereby arresting the genesis of stress related disorders. Pretreatment with drug extract has resulted in the increase in MAO activity above normal control values (Table 2), thereby decreasing the elevated levels of 5-HT and 5-HIAA induced by stress. Thus the antistress activity of these plant drugs could be attributed to the modulation of this enzymatic activity.

Table No-1 Free radical scavenging activity of Mussaenda frondosa extract

Extract concentration (µg/ml)	**Inhibition (%)**			
	DPPH	**Nitric oxide**	**Super-oxide**	**Hydroxyl radical**
1000	94.16±3.61	92.44±3.82	96.45±2.15	91.38±4.95
500	92.32±4.75	88.65±5.40	93.46±4.24	88.54±5.57
250	87.54±3.62	80.11±3.23	85.42±4.23	84.37±5.53
125	83.22±2.55	70.77±4.71	72.99±3.34	75.43±3.21
62	54.84±3.73	54.23±5.34	68.38±2.74	61.76±4.33
32	42.33±4.32	46.48±4.50	55.57±3.85	40.54±3.83
16	13.62±3.64	27.64±3.15	44.65±3.81	31.55±2.43
10	3.46±1.42	5.78±1.52	32.63±1.39	7.13±1.68
7	1.02±0.31	3.11±0.50	16.04±1.26	4.34±1.25
5	0.03±0.01	1.36±0.10	5.21±1.05	1.23±0.33
Ascorbic acid (100 µg)	95.11±4.22	85.34±4.11	87.32±5.87	94.44±4.71
BHT(20 µg)	92.27±3.31	-	-	-
Curcumin	-	91.7±3.11	-	-
IC_{50}	51.3±1.52	53.2±2.14	24.6±1.34	52.7±2.66

Values are expressed as mean ± S.E.M of 3 replicates

Table No-1 Effect of ethanolic extract of *Mussaenda frondosa* on brain bioamine and mao levels following cold immobilization stress.

Treatment group	Noradrenaline (ng/gm)	Dopamine (ng/gm)	5-Hydroxy trypyamine (ng/gm)	5-Hydroxy indoleacetic acid (ng/gm)	Monoamine oxidase (units/ mg)
Normal control	433.39±20.63	846.02±22.98	652.39±26.78	510.16±50.22	4.87±0.25
Restraint control	382.30±27.15	673.20±64.52	743.47±64.33	723.22±18.65	4.29±0.35
EEMF (500mg /kg)	521.99±61.02***	887.82±58.32***	397.16±20.88****	568.23±44.29**	7.88±0.23

n=6, Values are expressed as mean ± SEM

*p<0.05;**p<0.02;***p <0.01;****p<0.001, compared to the restrain control.

EEMF=Ethanolic extract of Mussanenda frondosa

Conclusion

Free radical induced per-oxidation has gained much importance because of their involvement in several pathological conditions such as cancer, cardiovascular disease, liver, diabetes milletus, inflammation, renal failure, brain dysfunction and stress among others. Healthy cells can scavenge free radicals effectively by means of antioxidants.

This study provides the evidence that free radical scavenging potential possessed by ethanolic extract of Mussaenda frondosa in vitro in different models and may be due to the presence of flavanoids reported.

The remarkable results are in agreement with normalizing affect of Mussaenda frondosa in rats against a variety of stressors, thereby indicating its adaptogenic potential. Further studies are in progress to identify active principle(s) responsible for its antistress activity and to find out synergy among different compounds present in Mussaenda frondosa.

References

1. Seyle H, The Evolution of Stress Concept. Am. Sci. 1973; 61: 692-699.
2. Rice ECA, Else. Sci, 1994; 131-53.
3. Khare CP, Indian medicinal plants- An illustrated dictionary, Springer, 2007; 295: 471.
4. Misra HP, Fridovich I. The univalent reduction of oxygen by reduced flavins and quinones. J Biol Chem 1972; 247: 188-92
5. Halliwell B, Gutteridge JMC, Aruoma OI, The deoxyribose method: a simple 'test tube' assay for determination of rate con-stants for reaction of hydroxyl radicals , Anal Biochem 1987; 165: 215-9.
6. Blois MS, Antioxidant determination by the use of a stable free radical. Nature 1958; 181: 1199-200.
7. Marcocci L, Maguire JJ, Droy- Lefaix MT, Packer L. The nitric oxide scavenging properties of Ginkgo biloba extract EGb 761. Boichem Biophys Res Commun 1994 ; 201 : 748-55.
8. Padma P, Khosa RL, Chansauria JPN, Ray AK. Effect of annona muricata and polyalthia cerasoides on brain neurotransmitters and enzyme monoamine oxidase following cold immobilization stress. J. Nat. Remed 2001 ; 2 : 144-146.
9. Ansell GB, Beeson MF. A rapid and sensitive procedure for the combined assay of noradrenaline, dopamine and serotonin in a single brain sample. Anal.Biochem. 1968; 23: 196-200.
10. Cox RH, Perhach JL. A sensitive, rapid and simple method for the simultaneous spectrophotofluorimetric determinations of norepinephrine, dopamine, 5- hydroxytryptamine and 5-hydroxyindoleacetic acid in discrete areas of brain. J. Neurochem, 1973; 20: 1777-1780.