



ADAPTOGENIC ACTIVITY OF ASPERAGUS RECEMOSUS AND EMBLICA OFFICINALIS

Rohit Saraswat, Pradeep Kumar Choda*

¹Professor, School of pharmacy, OPJS University, Churu, Rajasthan-331303

²Research scholar, School of pharmacy, OPJS University, Churu, Rajasthan-331303

*Corresponding author E-mail: pharmacologypradeep@gmail.com

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ABSTRACT

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Many plant species have been used by most ethnic groups for treating different disease conditions ranging from small infections to skin diseases, asthma, dysentery, malaria and other multiple indications. Herbal Medicine is usage of herbs for therapeutic and medicinal importance. A herb is a variety of chemical substances that act upon the body. Medicinal plants have been identified and utilized allover human history. Plants have the capacity to synthesize a plethora of chemical compounds that are used to normalize altered important biological functions. Stress is a physical and psychological response to perceived demands and pressures from within or from outside. To respond to these demands and pressures we mobilize physical and emotional resources. The hypothalamus pituitary adrenal axis plays a vital role in handling the stress. *Emblca officinalis* and *Asparagus racemosus* are widely used in the Ayurveda and are believed to increase defense against diseases. Pretreatment with *Emblca officinalis* and *Asparagus racemosus* extracts significantly reverted back reserpine induced ptosis and catalepsy. *Emblca officinalis* and *Asparagus racemosus* extracts in the plus maze, open field and light dark model test indicate that the effect detected is not false negative and that indeed both the extracts possess anxiolytic and anti-stress activity. *Emblca officinalis* and *Asparagus racemosus* extracts prevented the rise in plasma glucose due to its protective effect on adrenal gland resulting in lesser release in corticosteroids and glucocorticoids

INTRODUCTION

Herbal medicines are currently in demand and their popularity is increasing day by day. The use of herbal medicine becoming popular due to certainly not toxicity and side effects than allopathic medicines. This led to sudden increase in the number of herbal drug manufactures. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a

Great contribution towards maintaining human health^{1,2,3}. India is the largest producer of medicinal herbs and approximately called the botanical garden of the world. In Indian medicinal systems the most practitioners formulate and dispense their own recipes; hence, this requires proper documentation and research. Currently plant based drugs are researched and formulated in modern framework of medicine rather than in the form of galenic preparations or conventional dosage. The Principle of Phytotherapy is the

medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species. Plant metabolites: primary metabolites and secondary metabolites^{4,5}. Stress is a physical and psychological response to perceived demands and pressures from within or from outside. Too frequent, extreme or prolonged mobilization strains the body and generates the distress signals. These signals are experienced in a variety of ways often in the form of irritability, anger, anxiety, depression, fatigue, tension, headaches, stomachache, hypertension, migraine, ulcers, heart attack or colitis. Stress is both additive and cumulative in its negative effects on the individual and the society^{6, 7}. *Embllica officinalis* (EO) primarily contains tannins, alkaloids, phenolic compounds, amino acids and carbohydrates. It was studied against the cold stress-induced alterations in the behavioral and biochemical abnormalities. It is one of the myrobalans (plants that exhibit various therapeutic properties) commonly used in triphala (three fruits) preparations. Triphala is a traditional Ayurvedic herbal formulation which is considered an important rasayana in Ayurvedic medicine⁸. *Asparagus racemosus* (AR) is an important medicinal plant and its root paste or root juice has been used in various ailments and as health tonic well known Ayurvedic rasayana which prevent ageing, increase longevity, impart immunity, improve mental function, vigor and add vitality to the body and it is also used in nervous disorders, dyspepsia, tumors, inflammation, neuropathy, hepatopathy. Furthermore it has also been used to cure disorders of the nervous system, liver and certain infections⁹.

MATERIALS AND METHODS:

Chemicals like Methanol 70% Alcohol, n-Butanol, Pyridine, Acetonitrile and Benzyl bromide were obtained from Fine Chemicals, Hyderabad; reagents like Mayer's Reagent Dragendroff's Reagent, Hager's Reagent, Wagner's Reagent, Molisch's Reagent, Fehling's Reagent, Barfoed's Reagent, Benedicts Reagent, Ferric chloride, Lead acetate, Ninhydrin Reagent, Di-phenyl picrylhydrazyl (DPPH) Reagent obtained from Nikita Chemicals, Hyderabad.

In Vitro Antioxidant Activity: To the methanolic solution of DPPH an equal volume

of EO, AR extracts was added at different concentrations in methanol. Equal volume of methanol was added to control. Above mixture was kept for incubation at room temperature for 20 minutes. Absorbance was recorded at 517 nm¹⁰.

Nitric oxide scavenging activity: Sodium nitroprusside (5mM) in phosphate buffer saline was mixed with different concentrations of the EO, AR extracts and incubated at room temperature. A control without the test compound was taken. After incubation 1.5 ml of the incubation solution was removed and diluted with 1.5 ml of Griess reagent. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthyethelenediamine was measured at 546 nm¹¹.

Neuroprotective potential by thiobarbituric acid-reactive substances (TBARS) method:

EO, AR extracts were added to rat brain homogenate, was initiated by adding 100 µl of 15 mM FeSO₄ solution to 3 ml of brain homogenate. After 30 minutes of incubation at room temperature, 0.1 ml (10%w/v) of brain homogenate was taken in a tube containing 0.1 ml of SDS (8.1%w/v), 0.75 ml of 20% acetic acid and 0.75 ml of 0.8% Thiobarbituric acid (TBA) aqueous solution. The volume in each tube was made to 2 ml with distilled water and then heated on water bath at 95 degree Celsius for 60 minutes. After 60 minutes, the volume in each tube was made up to 2.5 ml and then 2.5 ml of n Butanol: Pyridine (5:1) was added in each tube. The reaction mixture was vortexed and centrifuged at 4000 rpm for 10 minutes. The organic layer was then removed and absorbance was read at 532 nm in a UV spectrophotometer¹².

In-Vivo Models

Measurement of Spontaneous Locomotor Activity by Activity Cage Model:

Test animals from various groups were pretreated with EO, AR at doses mentioned below. Diazepam used as a standard (i.e. positive control) was given orally 10 minutes prior to evaluation of its locomotor activity¹³. Groups like Group I- 0.1% Na CMC p.o. (Normal Control), Group II-Diazepam 2 mg/kg p.o (Positive Control), Group III-EO 100 mg/kg of

body weight *p.o.*, Group IV-EO 250 mg/kg of body weight *p.o.*, Group V-EO 500 mg/kg of body weight *p.o.*, Group VI-AR 100 mg/kg of body weight *p.o.*, Group VII-AR 250 mg/kg of body weight *p.o.* and Group VIII-AR 500 mg/kg of body weight *p.o.*

Potentiation of Sleep Time of Standard Reference Sedative: The control animals were given vehicle 45 minutes prior to the intraperitoneal (*i.p.*) injection of standard reference sedative, diazepam while the test group received the respective oral doses of EO, AR as mentioned. 45 minutes prior to thiopentone of 50mg/kg was administration to all groups, animals showed extension of hind limb; became immobile and sedated indicating onset of sleep on diazepam treatment¹¹.

Evaluation of Motor-Coordination by Rota-rod test: extracts were orally administered, only to those mice which demonstrated the ability to remain on the rod rotating at 25 rpm for at least 150 seconds, were included in the test. While for testing the effect of extracts administered orally at various doses, the parameters to be fulfilled were set at ability to remain on the rod for 60 seconds at 20 rpm. After 18 hours of fasting, the mice were administered the extracts at various doses and evaluated for skeletal muscle relaxation on rotarod apparatus. Here lower dose is not included like 100mg/kg as they did not show any significant CNS stimulant or depressant action¹⁰.

Anti -Depressant Activity

Despair Swim Test: Sixty minutes after administration of the EO, AR, and male mice were placed in plexiglass cylinder/ plastic tub containing 9 cm of water maintained at 25°C. After allowing one minute for acclimatization, immobility of each mouse was rated every 30s from second minute onwards for another five minutes. Standard drug Ginseng (100 mg/kg) and reserpine (5mg/kg) were chosen as positive control and negative control agents respectively for the experiment¹³. Experimental groups are broadly as follows: Group I- 0.1% Na CMC *p.o.* (Normal Control), Group II- Ginseng 100mg/kg *p.o.* (Positive Control), Group III- Reserpine 5mg/kg *i.p.* (Negative Control), Group IV-EO 100 mg/kg of body weight *p.o.*, Group V-EO 250 mg/kg of body weight *p.o.*, Group VI-EO 500 mg/kg of body weight *p.o.*,

Group VII-AR 100 mg/kg of body weight *p.o.*, Group VIII- AR 250 mg/kg of body weight *p.o.*, Group IX- AR 500 mg/kg of body weight *p.o.*

Tail Suspension Test in Mice: For the test the mice were suspended on the edge of a shelf 58 cm above a tabletop by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for a period of 5 minutes. Mice were considered to be immobile when they hung passively and completely motionless¹⁴.

Reserpine Antagonism in Mice: Sixty minutes after oral or 30 minutes after *i.p.* administration of the test compound or the vehicle, 5mg/kg reserpine was injected *s.c.* The test started 150 minutes after reserpine administration was continued for 2hrs. Catalepsy and ptosis were used as criteria for evaluation¹⁵.

Anti-Stress Activity

Determination of Norepinephrine: Brain was dissected out and stored at -10°C. The whole brain was homogenized in a mixture of 2 ml of 0.1N HCl and 10 ml butanol in a Glass Teflon homogeniser. 5 gm of sodium chloride was added to the brain homogenate and stirred for 60 minutes. Butanol layer was removed and added to a mixture of 50ml n-heptane+7ml 0.1N HCl. This mixture was then shaken for 5 minutes to extract amines from organic to aqueous phase. The aqueous phase/acid extract was recovered by centrifugation at 3000 rpm for 5 min and used for nor-epinephrine by using fluorimetric assays¹². Animals were grouped as follows: Group I- 0.1% Na CMC *p.o.* (Normal Control), Group II-Ginseng 100 mg/kg *p.o.* + chronic restraint stress (Positive Control), Group III- 0.1% Na CMC *p.o.*+ chronic restraint stress (Stress Control), Group IV-EO 250 mg/kg of body weight *p.o.* + chronic restraint stress, Group V-EO 500 mg/kg of body weight *p.o.*+ chronic restraint stress, Group VI- AR 250 mg/kg of body weight *p.o.* + chronic restraint stress, Group VII-AR 500 mg/kg of body weight *p.o.*+ chronic restraint stress.

Effect of chronic stress on working memory by elevated plus maze: All the Male Swiss albino mice were pretreated for 14 days with the respective test drugs. At the end of the 14 days rats of different groups were exposed to

stressful stimuli for further seven days. At the end of 21st day the mice were subjected to the plus maze¹⁶. The Elevated plus maze apparatus used for the study was similar to that described for the Anxiolytic study. Experimental Groups as follows: Group I-0.1% Na CMC p.o. (Normal Control), Group II- Ginseng 100 mg/kg p.o + chronic restraint stress (Positive Control), Group III-0.1% Na CMC p.o.+ chronic restraint stress (Stress Control), Group IV- EO 100 mg/kg of body weight p.o. + chronic restraint stress, Group V- EO 250 mg/kg of body weight p.o. + chronic restraint stress, Group VI-EO 500 mg/kg of body weight p.o.+ chronic restraint stress, Group VII-AR 100 mg/kg of body weight p.o. + chronic restraint stress, Group VIII-AR 250 mg/kg of body weight p.o. + chronic restraint stress, Group IX- AR 500 mg/kg of body weight p.o.+ chronic restraint stress.

RESULT AND DISCUSSION:

In Vitro Antioxidant Activity: *In vitro* antioxidant activity of the plant extracts was observed by DPPH method. DPPH activity of extracts of EO and AR is presented in Fig.No.1. Extracts of EO and AR have got profound antioxidant activity. Results have proven the effectiveness of the Methanolic, and aqueous extracts compared. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. All the extracts of EO and AR exhibited a significant dose-dependent inhibition of DPPH activity.

Nitricoxide scavenging activity: The results of NO scavenging activity of the selected plant extracts are shown in Table 1. Nitricoxide or reactive nitrogen species, formed during the irreaction with oxygen or with superoxides, such as NO_2 , N_2O_4 , N_3O_4 , NO_3 and NO_2 are very reactive. These compounds are responsible for altering the structural and functional behaviour of many cellular components.

Phenolic compounds and flavonoids have been reported to be associated with anti-oxidative action in biological systems, acting as scavengers of single toxygen and freeradicals. The nitricoxide scavenging activity of flavonoids and phenolic compounds are known, we can speculate that these constituents might be responsible for the observed nitricoxide scavenging activity.

Neuroprotective potential by thiobarbituric acid-reactive substances (TBARS) method:

In the present study, our results indicated that increased the levels of thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation. TBARS is an end product of lipid peroxidation, a measure of free radical generation. It has been previously reported that the elevation of TBARS is due to an oxidative injury by hydroxyl radicals. Although the levels of TBARS were enhanced induced neurotoxicity, EO and AR extracts decreased these levels in a significant manner.

In- Vivo Models

Measurement of Spontaneous Locomotor Activity by Activity Cage Model:

Pretreatment with EO hydro alcoholic extract at the dose of 250 and 500mg/kg significantly increased spontaneous locomotor activity in mice compared to vehicle treated mice. Increased spontaneous locomotor activity is may be due to CNS stimulant action. Pretreatment with AR extracts at the dose of 100, 250 and 500 mg/kg did not produce significant changes in spontaneous locomotor activity in mice as compared to diazepam treated mice. Therefore AR has no CNS stimulant action.

Potentiation of Sleep Time of Standard Reference Sedative:

Pretreatment with EO did not potentiate the thiopentone induced duration of sleep. Therefore, EO has no CNS depressant activity. Pretreatment with AR hydroalcoholic extract at the doses of 500mg/kg significantly increased the thiopentone induced duration of sleep in mice. Increase in sleep duration is correlated with CNS depressant action.

Evaluation of Motor Coordination by Rota-rod test:

Pretreatment with EO, AR did not exhibit any effect on motor coordination as determined by Rota-rod test.

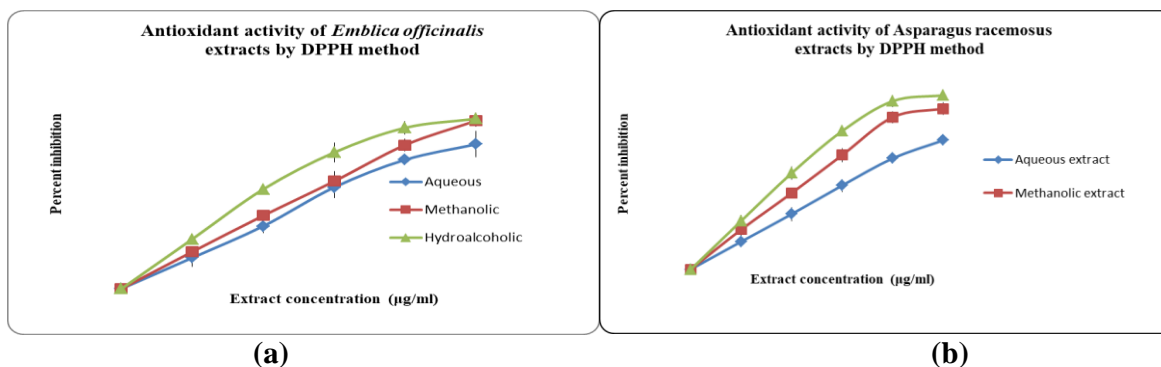


Fig. No.1 Antioxidant activity by DPPH method from extracts of (a)-EO (b)- AR
Table No. 1: Nitric oxide scavenging activity of *Emblica officinalis* and *Asparagus racemosus* extracts

Parameters	<i>E o</i> IC ₅₀ values (µg/ml)	A RIC ₅₀ values (µg/ml)
Aqueous extract	25.20	266.69
Methanolic extract	22.34	267.85
Hydro alcoholic extract	20.66	238.00
Ascorbic acid	15.32	15.32

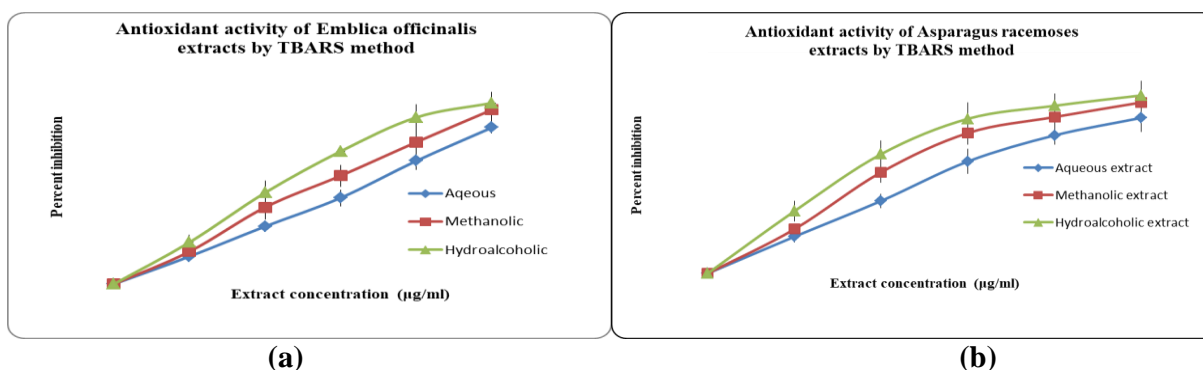


Fig. No2. Antioxidant activity by TBARS method from extracts of (a)-E O (b)- A R

Table No 2: Spontaneous locomotor activity in mice of *Emblica officinalis* and *Asparagus racemosus*

Groups	Dose mg/kg p.o	Points scored for 10 min ± S.D of E O	Points scored for 10 min ± S.D of A R
Aqueous extract	100	498±26.47	500±13.18
	250	511±30.11	485±23.7
	500	488±18.96	487±29.8
Methanolic extract	100	496±27.14	499±30.2
	250	494±36.4	475±15.6
	500	489±25.94	480±10.2
Hydroalcoholic extract	100	547±27.4	475±21.3
	250	600±15.66*	487±17.8
	500	650±21.67*	507±16.6
Normal	-	493±22.13	493±22.13
Diazepam	2	298±31.21**	298±31.21**

N=6, values are mean ± S.D; *P < 0.05, **P < 0.01, when compared to normal group by one-way ANNOVA followed by Dunnett's test

Table No 3: effect of *Emblica officinalis* and *Asparagus racemosus* on thiopentone induced sleep in mice

Groups	Emblica officinalis			Asparagus racemosus		
	Dose mg/kg p.o	Points scored for 10 min	±S.D	Dose mg/kg p.o	Points scored for 10 min	±S.D
Aqueous extract	100	7.12	2.14	100	8.16	1.34
	250	9.47	1.9	250	10.24	4.89
	500	10.23	2.7	500	11.46	2.67
Methanolic extract	100	8.97	1.89	100	12.31	3.67
	250	12.23	1.44	250	10.24	2.56
	500	7.23	2.4	500	11.34	3.64
Hydroalcoholic extract	100	9.78	3.1	100	14.6	3.1
	250	10.31	3.45	250	16.13	2.78
	500	8.77	1.7	500	17.26*	1.22
Normal	-	9.446	1.3	-	9.446	1.3
Diazepam	2	40.78**	4.5	2	40.78**	4.5

N=6, values are mean ± S.D; *P< 0.05, **P < 0.01, when compared to normal group by one-way ANNOVA followed by Dunnett's test

Table No 4: Pretreatment with *Emblica officinalis*, *Asparagus racemosus*

	Normal	EO		AR	
		250 mg	500 mg	250 mg	500 mg
Time spent on the rod (Sec.)	300	298	298	287	276
S.D	10.27	14.53	10.22	11.47	12.51

N=6, values are mean ± S.D; *P< 0.05, **P < 0.01, when compared to normal group by one-way ANNOVA followed by Dunnett's test

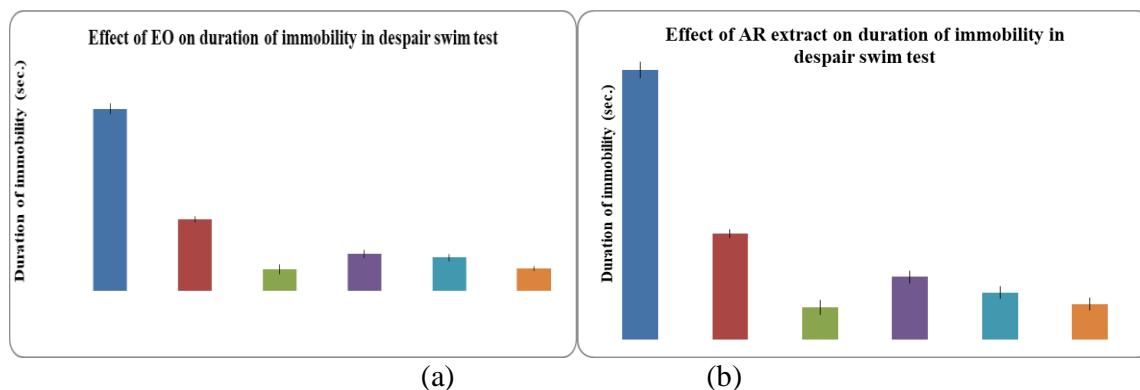


Fig. No.3: Effect on duration of immobility in despair swim test of extracts (a)- EO (b)-AR

Table No 5: effect of EO and AR hydroalcoholic extract on tail suspension test

Duration of Immobility (s)	Reserpine 5mg/kg	Normal	Ginseng 100 mg/kg	EO			AR		
				100 mg	250 mg	500 mg	100 mg	250 mg	500 mg
	173.3**	76.16	50.2*	70.36	74.23	56.32*	75.2	68.3	58.74*
S.D	17.23	6.8	4.67	5.97	4.12	3.78	6.23	3.14	4.33

N=6, values are mean ± S.D; *P< 0.05, **P < 0.01, when compared to normal group by one-way ANNOVA followed by Dunnett's test

Table No 6: Effect of EO and AR extracts on % scores of reserpine induced ptosis and catalepsy in mice

	Normal	Ginseng 100mg/kg	EO			AR		
			100 mg	250 mg	500 mg	100 mg	250mg	500 mg
Degree of ptosis %	100	6.25**	43.75	31.25*	12.5**	43.75	12.5	6.25**
Degree of catalepsy %	100	68.75*	87.5	62.5*	50*	75*	62.5*	43.75**

N=6, values are mean \pm S.D; *P< 0.05, **P < 0.01, when compared to normal group by one-way ANNOVA followed by Dunnett's test

Table No 7: Chronic stress induced brain Nor-epinephrine level

	Normal	Stress control	Ginseng 100mg	EO250	EO500	AR250	AR500
NE μ g/gm	28.7*	40.20	30.24*	37.8	35.41*	37.41	34.73*
S.D	1.3	2.47	1.06	1.7	0.67	2.31	2.4

N=6, values are mean \pm S.D; *P< 0.05, **P < 0.01, when compared to stressed group by one-way ANNOVA followed by Dunnett's test.

Table No 8: Effect of EO and AR Hydroalcoholic extract on working memory in elevated plus maze

Treatment	Latency to reach the closed arm in sec of EO		Latency to reach the closed arm in sec of AR	
	Day 1	Day 2		
Normal	28.9 \pm 7.01	20.42 \pm 6.90*	28.9 \pm 7.01	20.42 \pm 6.90*
Stress Control	42.87 \pm 2.41	40.22 \pm 3.41	41.72 \pm 5.70	40.0 \pm 2.74
EO100 mg/kg p.o.	44.12 \pm 6.60	41.25 \pm 4.94	39.81 \pm 3.42	37.33 \pm 4.77
EO 250 mg/kg p.o.	34.64 \pm 3.59	32.41 \pm 5.17	39.39 \pm 5.37	36.43 \pm 3.67
EO 500 mg/kg p.o.	42.31 \pm 4.44	21.72 \pm 2.9*	30.34 \pm 8.65	17.75 \pm 6.23*
Ginseng 100mg/kg p.o	30.34 \pm 8.65	17.75 \pm 6.23*	28.9 \pm 7.01	20.42 \pm 6.90*

N=6, values are mean \pm S.D; *P< 0.05, **P < 0.01, when compared to stressed group by one-way ANNOVA followed by Dunnett's test.

Based on the preliminary studies hydroalcoholic extracts of EO showed CNS stimulant activity. Hydroalcoholic extracts AR at higher doses may possess CNS depressant action. Hydroalcoholic extracts of EO, AR were further screened for anti-depressant like activity.

Anti -Depressant Activity

Despair Swim Test: Like Ginseng, EO hydroalcoholic extract (100, 250mg/kg and 500 mg/kg) significantly reduced mean immobility time in mice as compared to normal control. Like Ginseng, AR hydroalcoholic extract (100, 250mg/kg and 500 mg/kg) significantly reduced mean immobility time in mice as compared to normal control.

Tail Suspension Test in Mice: Like Ginseng, EO hydroalcoholic extract at 500 mg/kg significantly reduced mean immobility time in mice as compared to normal control. AR hydroalcoholic extract (500 mg/kg) significantly reduced mean immobility time in mice as compared to normal control.

Reserpine Antagonism in Mice: Pretreatment with EO and AR extract (250mg/kg and 500 mg/kg) significantly reverted reserpine induced ptosis in mice as indicated by reduced scores of degree of ptosis. Further, EO treatment significantly reduced the catalepsy scores as compared to normal control.

Anti-Stress Activity

Determination of Norepinephrine: Pretreatment with EO, AR hydroalcoholic extracts at the dose of 500mg/kg significantly reduced the brain Nor-epinephrine levels as compared to stressed rats.

Effect of chronic stress on working memory by elevated plus maze: Stressed mice took longer time to reach the closed arm. However Ginseng treatment (100mg/kg) significantly reduced the time required to find the closed arm. It improved basal performance with respect to acquisition and retention of memory. Hydroalcoholic extracts of EO significantly improved the latency to reach the closed arm. Therefore EO at the dose of 500mg/kg alleviated the stress induced poor cognition in mice.

CONCLUSION:

From the results it was evident that pretreatment with EO extracts did not potentiate duration of sleep induced by thiopentone sodium. Therefore EO has no CNS depressant activity. AR Hydroalcoholic extract at the dose of 500mg/kg increased the thiopentone induced sleep time significantly. Results obtained suggest that EO, AR extracts may be useful as a therapeutic drug in dealing with stress related problems. In the Rota rod test animals treated with EO, AR extracts performed similarly to control suggesting that extracts did not affect motor coordination of animals. The reduction of immobility time in swimming despair test revealed antidepressant like potential of EO, AR. Further, pretreatment with these extracts significantly reverted back reserpine induced ptosis and catalepsy in mice as evidenced by reduced scores of degree of ptosis and catalepsy respectively. Results obtained in the plus maze, open field and light dark model test indicate that the effect detected is not false negative and that indeed both the extracts possess anxiolytic and anti-stress activity. So, it was concluded that the fruit extract of *Emblca officinalis* & leaf extract of *Asperagus recemosus* have anti stress activity.

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