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ANTISTRESS ACTIVITY OF *DIOSPYROS MALABARICA* (DESR.) KOSTEL IN MICE AND RATS

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ABSTRACT: The purpose of this study was to evaluate antistress activity of roots of *Diospyros malabarica* (Desr.) Kostel (DMDK) against anoxia stress tolerance, chemical induced stress, swimming endurance stress and immobilisation stress models in mice and rats. Ethanolic extract of roots of DMDK (EERDMDK) was screened for its antistress activity at 125 mg/kg, 250 mg/kg, 500 mg/kg, p.o. doses and diazepam was selected as reference standard at 2 mg/kg i.p. dose. Stress was induced by anoxia stress tolerance, chemical induced, swimming endurance and immobilisation stress models in mice and rats. The antistress activity was evaluated by measuring anoxia stress tolerance time, number of writhes, immobility time, organs weight of animals and estimating biochemical parameters such as glucose, cholesterol and blood urea nitrogen. Pretreatment with EERDMDK showed significant increases in anoxia stress tolerance time and significant decreases in number of writhes and immobility time in mice and this effect was dose dependent when compared to control. In immobilisation stress model pretreatment with EERDMDK showed significant reduction in level of biochemical parameters such as glucose, cholesterol and blood urea nitrogen, whereas stress induced increase in liver, adrenal gland weight and decrease in weight of spleen were significantly reversed by EERDMDK in dose dependent manner i.e. EERDMDK restored weight of organs and level of biochemical parameters when compared to positive control. The conclusion of our study indicated that EERDMDK possessed significant antistress activity and possible mechanism underlying this effect is mediated through normalization of catecholamines level.

INTRODUCTION: Stress is a common phenomenon that is experienced by every person. When stress is extreme it is dangerous to body and, hence needs to be treated. Stress is involved in the pathogenesis of a variety of diseases like psychiatric disorders such as anxiety and depression, immunosuppression, male impotence, peptic ulcer, diabetes mellitus [1]. Stress has been shown to induce a marked increase in the brain levels of biogenic amines such as adrenaline and nor-adrenaline [2].

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These biogenic amines are releases in response to stress signals and are meant to assist the organisms to cope with stress [3]. There is no perfect treatment in the modern drug therapy for stress related diseases. The available techniques for increasing endurance performance include physical training for endurance work, yogic and medication practices, supplementation of nutraceuticals and intervention of adaptogens.

The term Adaptogens means an agent that improves adaptation capacity of the organism during stress and Antistress (Pharmacological word for the same) means an agent which nullifies or prevents ill effects of stress and improves adaptation [4]. The potential utility of safer and cheaper herbal medicines as antistress agents have been reported in literature as antistress agents [5, 6].

Several plants are known to exhibit credible medicinal properties for the treatment of stress and need to be explored to identify their potential application in prevention and therapy of human ailments. *Diospyros malabarica* (Desr.) Kostel (DMDK), family – Ebenaceae, is a tree distributed throughout India. The DMDK has been used since ancient time in Indian folklore medicine for various clinical conditions such as liver diseases, snake bites, diabetes, diarrhea, urinary diseases and renal stone [7, 8, 9, 10]. The plant possesses flavonoids, tannins, terpenoides, sugars, diospyrin, napthoquinones, hydrocarbons and steroids [11]. Although, the antiurolithiatic [12], and antioxidant [13] properties of DMDK have been reported. Thus, the present study was designed to assess the antistress ability of roots of DMDK against anoxia stress tolerance, chemical induced stress, swimming endurance stress and immobilisation stress models in mice and rats.

MATERIALS AND METHODS:

Plant material:

Roots of DMDK was collected in bulk quantities from our college campus area and authenticated by Department of Botany, S.S.M.M. Baramati. The roots of DMDK were washed with distilled water to remove dirt and soil. It was further shade dried and then coarsely powered.

Preparation of extract:

The powder of roots of DMDK was subjected to successive soxhlet extraction with solvents of increased polarity. The ethanolic extract was selected for the present study. The extract was concentrated using rotary flash evaporator and stored at room temperature.

Preliminary Phytochemical study:

Preliminary Phytochemical screening was carried out on ethanolic extract of roots of *Diospyros malabarica* (Dser.) Kostel (EERDMDK) for detection of phytoconstituents present following the standard methods described in practical pharmacognosy book by Dr. C. K. Kokate [14] and K. R. Khandelwal [15].

Acute toxicity study:

Acute toxicity study of EERDMDK was performed on albino mice (20 – 30 g) maintained under standard conditions. Fixed dose method of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) was adopted for toxicity studies [16, 17] (OECD Guideline No. 420).

Animals:

Healthy, albino mice (20-30 g weight) and rats (150- 225 g) were used for the study. The mice and rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3 ºC and 35-60% humidity). They were feed with standard feed and water ad libitum. The study was approved by the Institutional Animal Ethical Committee of S.V.P.M's College of Pharmacy, Malegaon Bk II, Baramati, registered under CPCSEA, India (Registration No. 1214/ac/08/CPCSEA).

Evaluation of antistress activity:

Anoxia stress tolerance test in mice:

Albino mice of either sex weighing 20-30 g were selected and divided in to five groups of six each. Group I received only vehicle (1mL/kg, p.o.) and served as vehicle control. Group II EERDMDK (125 mg/kg, p.o.), Group III EERDMDK (250 mg/kg, p.o.), Group IV EERDMDK (500 mg/kg, p.o.) and Group V diazepam (2 mg/kg, i.p.). Animals were treated as shown above for the three weeks. At the end of 1st, 2nd and 3rd week i.e. on the 7th, 14th and 21st day one hour after the treatment stress was induced by placing each animal individually in the hermetic vessel of 1liter capacity to record anoxia tolerance time. The movement when the animal showed the first convulsions immediately removed from the vessel and resuscitated if needed. The time duration of entry of the animal in to the hermetic vessel and the appearance of the first convulsion was taken as time of anoxia tolerance. Appearance of convulsion was very sharp end point, as delay by minute of removal of the animal from vessel may lead to death of the same [18].

Chemical induced stress in mice:

Albino mice were randomly divided in to five groups of 6 animals each. Group I received distilled water (0.1 mL/100 g) and served as vehicle control. Animals in Group II, Group III and Group IV were treated with different doses (125, 250, 500 mg/kg

p.o.) of EERDMDK. Animals in Group V received diazepam (2 mg/kg, i.p.). All the treatments were given continuously for 15 days. On day 15, one hour after the drug treatment all the animals received 0.1 mL of 6% (v/v) glacial acetic acid i.p. and number of writhes was observed in all the groups for 20 min [19, 20].

Swimming endurance test in mice:

Albino mice were randomly divided in to five groups of 6 animals each. Group I received distilled water (0.1 mL/100 g) and served as vehicle control. Animals in Group II, Group III and Group IV were treated with different doses (125, 250, 500 mg/kg p.o.) of EERDMDK. Animals in Group V received diazepam (2 mg/kg, i.p.). All the treatments were given continuously for 10 days. On day 10, one hour after the drug treatment all the animals were allowed to swim individually in a glass tank filled with water. The immobility time of each mouse was recorded for 30 min [20].

Immobilisation stress in rats:

Adult male albino rats of 150-225 g were selected and divided in to six groups of six animals each as Group I Negative control (Unstressed, untreated), Group II Positive control (Stressed, received vehicle), Group III EERDMDK (125 mg/kg p.o.), Group IV EERDMDK (250 mg/kg p.o.), Group V EERDMDK (500 mg/kg p.o.), Group VI diazepam (2 mg/kg i.p.). The treatment was made stated above for 10 days 1 h before the exposure of stress. Stress was induced by immobilising rats with head down, supine position by fixing the forelimbs and hand limbs to a wooden board inclined at an angle of 600, daily 2 h for a period of 10 days. The animals were sacrificed at the end of specified period and blood was collected by retro-orbital for estimation of biochemical parameters such as serum glucose, cholesterol and blood urea nitrogen. The weight of organs, such as liver, spleen and adrenal glands after washing with alcohol was recorded per 100 g body weight of animal [21].

Statistical analysis:

The data were presented as men ± standard error of mean (SEM) and analyzed using one –way analysis of variance (ANOVA) followed by Dunnett's and $P< 0.05$ was considered statically significant. Statistical Package for Social Science (SPSS 20.0) version software was used for statistical analysis.

RESULTS:

Preliminary Phytochemical study:

The Preliminary Phytochemical investigation of EERDMDK indicated the presence of flavonoids, carbohydrates, tannins.

Acute toxicity study:

The EERDMDK was studied for acute toxicity at dose of 2000 mg/kg p.o. The extract was found to be safe and no mortality of the animals observed. Hence 2500 mg/kg was considered as LD_{50} cut off value as per fixed dose method of CPCSEA. So, the doses selected for the evaluation of antistress activity of EERDMDK were 125 mg/kg, 250mg/kg and 500mg/kg p.o.

Antistress activity:

Effect of EERDMDK on Anoxia stress tolerance test in mice:

Anoxia stress tolerance time was significantly enhanced on 7^{th}, 14^{th} and 21^{st} day in EERDMDK at doses 250 mg/kg ($P< 0.01$) and 500 mg/kg ($P< 0.001$), p.o. and diazepam at 2 mg/kg ($P< 0.001$) i.p. treated groups and effects was dose dependent manner. However the effect EERDMDK at 125 mg/kg dose on anoxia stress tolerance time in mice was not statically significant at the end of 1^{st}, 2^{nd} and 3^{rd} week of treatment. The results are shown in **Table 1**.

Effect of EERDMDK on Chemical induced stress in mice:

A glacial acetic acid 0.1mL of 6% (v/v) significantly increases the number of writhes in mice. Pretreatment with EERDMDK at 125 mg/kg ($P< 0.05$) and 250 mg/kg ($P< 0.01$) doses were found to be significantly inhibit number of writhes in 20 minutes when compared to vehicle treated group. However EERDMDK at high dose 500 mg/kg ($P< 0.001$) and diazepam at 2 mg/kg ($P< 0.001$) dose were found to be significantly inhibit number of writhes in 20 minutes when compared to vehicle treated group i.e. effect of EERDMDK was dose relative manner. The results are shown in **Table 2**.

Effect of EERDMDK on swimming endurance test in mice:

The immobility time was significantly increases in vehicle treated control group of animals; whereas pretreatment with EERDMDK at 125 mg/kg and 250 mg/kg doses were found to be significantly (P< 0.05) reduced immobility time in 30 minutes. However EERDMDK at high dose 500 mg/kg (P< 0.01) and diazepam at 2 mg/kg (P< 0.01) dose were found to be significantly reduced immobility time in 30 minutes. The results are shown in **Table 3**.

Effect of EERDMDK on Immobilisation stress in rats:

Effect on biochemical parameters:

The immobilisation stress caused marked increase in biochemical parameters like glucose, cholesterol and blood urea nitrogen in rats. Pretreatment with EERDMDK at 125mg/kg, 250 mg/kg p.o. doses were significantly (P< 0.05) reversed elevated levels of such biochemical parameters. However EERDMDK at high dose 500 mg/kg were significantly (P< 0.01) reversed elevated levels of such biochemical parameters i.e. effects was dose dependent manner. The results are shown in **Table4**.

Effect on organs weight:

The immobilisation stress caused marked changes in organs weight i.e. weight of spleen was significantly reduced and weight of liver and adrenal gland was significantly increased in rats. However EERDMDK at, 125 mg/kg and 250 mg/kg p.o. doses were significantly (P< 0.05) significantly increased weight of spleen and decreased weight of liver and adrenal gland. Whereas treatment with EERDMDK at high dose i.e. 500 mg/kg p.o. were significantly (P< 0.01) increased weight of spleen and decreased weight of liver (P< 0.001) and adrenal gland (P< 0.01) i.e. effects was dose dependent manner. The results are shown in **Table 5**.

TABLE 1: EFFECT OF EERDMDK ON ANOXIA STRESS TOLERANCE TIME IN MICE.

Groups	Duration of anoxia stress tolerance (min.)		
	7th Day	14th Day	21st Day
Control (Vehicle)	133.33± 1.52	134.50±1.65	135.17±1.46
EERDMDK 125 mg/kg	136.63±1.56	140.66±1.73	142.23±1.63
EERDMDK 250 mg/kg	149.98±1.28**	153.40±1.92**	161.63±2.56**
EERDMDK 500 mg/kg	163.17±2.37***	166.20±1.71***	170.97±1.95***
Diazepam 2 mg/kg	168.02±1.20***	170.77±1.35***	174.50±2.22***

Values are expressed as mean ± SEM, n=6, *P<0.05, **P<0.01, ***P<0.001 compared with control (one –way ANOVA followed by Dunnett's test).

TABLE 2: EFFECT OF EERDMDK ON CHEMICAL INDUCED STRESS IN MICE.

Groups	Number of writhes (min.)
Control (Vehicle)	50±1.52
EERDMDK 125 mg/kg	38±2.37*
EERDMDK 250 mg/kg	29±2.52**
EERDMDK 500 mg/kg	13±1.52***
Diazepam 2 mg/kg	9±1.60***

Values are expressed as mean ± SEM, n=6, *P<0.05, **P<0.01, ***P<0.001 compared with control (one –way ANOVA followed by Dunnett's test).

TABLE 3: EFFECT OF EERDMDK ON SWIMMING ENDURANCE TEST IN MICE.

Groups	Immobility time (min.)
Control (Vehicle)	26.00±0.75
EERDMDK 125 mg/kg	21.52±0.36*
EERDMDK 250 mg/kg	20.60±0.78*
EERDMDK 500 mg/kg	18.41±0.87**
Diazepam 2 mg/kg	17.10±0.72**

Values are expressed as mean ± SEM, n=6, *P<0.05, **P<0.01, ***P<0.001 compared with control (one –way ANOVA followed by Dunnett's test).

TABLE 4: EFFECT OF EERDMDK ON BIOCHEMICAL PARAMETERS IN IMMOBILISATION STRESS IN RATS.

Groups	Biochemical estimation (mg/dl)		
	Glucose	Cholesterol	Blood urea nitrogen
Negative Control (Unstressed)	81.09±2.86	55.42±2.53	21.29±1.21
Positive Control (Stressed, Vehicle)	133.51±3.36	86.62±3.05	44.41±3.13
EERDMDK 125 mg/kg	113.39±2.50*	70.70±2.13*	29.94±1.22*
EERDMDK 250 mg/kg	101.38±5.42*	64.72±1.83*	28.29±1.59*
EERDMDK 500 mg/kg	92.09±3.60**	60.16±2.78**	23.04±1.21**
Diazepam 2 mg/kg	87.91±2.67***	58.59±2.43**	22.26±1.35**

Values are expressed as mean ± SEM, n=6, *P<0.05, **P<0.01, ***P<0.001 Compared with positive control (one –way ANOVA followed by Dunnett's test).

TABLE 5: EFFECT OF EERDMDK ON ORGANS WEIGHT IN RATS.

Groups	Organs weight (gm/100 gm body weight)		
	Liver	Adrenal gland	Spleen
Negative Control (Unstressed)	3.74±0.097	0.014±0.001	0.42±0.017
Positive Control (Stressed, Vehicle)	5.22±0.076	0.033±0.002	0.24±0.013
EERDMDK 125 mg/kg	4.74±0.054*	0.017±0.001*	0.36±0.024*
EERDMDK 250 mg/kg	4.35±0.045**	0.018±0.001*	0.38±0.016**
EERDMDK 500 mg/kg	4.09±0.069***	0.013±0.001**	0.39±0.014**
Diazepam 2 mg/kg	3.96±0.064***	0.014±0.001**	0.40±0.014**

Values are expressed as mean ± SEM, n=6, *P<0.05, **P<0.01, ***P<0.001 Compared with positive control (one –way ANOVA followed by Dunnett's test).

DISCUSSION: In the present investigation EERDMDK has been evaluated for the antistress activity against different types of stress i.e. Anoxia stress tolerance, Chemical induced stress, swimming endurance and Immobilisation stress models. Diazepam was used standard drug because there are report that diazepam possess a non specific antistress activity involving the mesocortical dopamine system and the norepinephrine and 5 HT levels of whole brain and hypothalamus and it is proposed that this effect is might be due to enhancement of GABAergic neurotransmission [22].

In anoxia stress tolerance model, depletion of oxygen in hermetic vessel leads to convulsions in animals and pretreatment with EERDMDK had increased the duration of stress tolerance indicating their antistress activity. The possible mechanism may be due to that during stress, EERDMDK was capable of increasing succinate dehydrogenase level in brain and this enzyme is responsible for utilization and conservation of energy in the cellular system of the organisms, which helps adaptive process during stress. In case of swimming endurance model EERDMDK exhibited significant antistress activity as indicated by significant reduction of immobility time in animals. There are reports that plasma levels of adrenaline and noradrenaline are elevated and monoamine oxidase level decrease in the brain during stress induced by swimming endurance test [23]. The possible mechanism of antistress activity EERDMDK against swimming endurance model might be due to the normalization of catecholamines and monoamine oxidase level. In immobilisation stress model significant increase in biochemical parameters such as glucose, cholesterol and blood urea nitrogen in positive control group when compared to the other groups.

In the present investigation significant hyperglycemia was observed and there are reports that under stressful conditions cortisol in human and corticosterone in rats will be secreted by adrenal cortex and hyper secretion of cortisol helps the maintenance of internal homeostasis by the process of gluconeogenesis and lipogenesis [23].

EERDMDK significantly reduced hyperglycemia might be due to by reducing hyperactivity of adrenal cortex and maintenance of homeostasis mechanism in immobilisation stress model. The mechanism by which stress increases serum cholesterol is likely to be related to the enhanced activity of hypothalamo-hypophyseal axis (HPA) resulting in liberation of catecholamines and corticosteroids. This could lead to increase in blood cholesterol level science epinephirine is known to mobilise lipids from adipose tissues. The increase

in release of catecholamines leads to elevated levels of glucose and blood urea nitrogen [24].

In immobilisation stress model EERDMDK significantly reduced elevated levels of glucose, cholesterol and blood urea nitrogen in dose dependent manner and the possible mechanism may be due to the normalization of catecholamines level. In immobilisation stress model weight of spleen was significantly decreased and weight of liver and adrenal gland was found to be significantly increased. There are reports that weight of liver and adrenal gland was increases due to cortisol increases mRNA levels in liver result in increases weight of liver, whereas stress induces adreno –medullary response in man and adrenaline in turns stimulates β receptors on the pituitary glands causing greater release of ACTH, which can stimulate the adrenal medulla as well as cortex result in increases weight of adrenal gland. In addition there are reports that spleen constrict to release more blood cell (RBC) during stress and result in decreases weight of spleen [25, 26].

This stress induced changes in organs weight were significantly reversed by the EERDMDK in dose dependent manner i.e. EERDMDK restored organs weight of animals. In case of chemical induced stress model number of writhes was significantly increased in control group. There are reports that glacial acid induced stress test caused hyperalgesic effects on the pain pathway [27].

However pretreatment with EERDMDK was found to be significantly reduces number of writhes in dose dependent manner. The possible mechanism for reduction in number of writhes by EERDMDK might be due to the inhibition of pain and inflammatory processes. All these findings confirmed that antistress activity of EERDMDK was significant and dose dependent manner. Literature survey indicates that flavonoids, triterpenes and tannins were reported to possess variety of pharmacological action including antistress activity. In the present investigation also preliminary phytochemical screening on EERDMDK gave positive test for flavonoids and tannins, this might be the reason for significant antistress activity of EERDMDK.

CONCLUSION: From the obtained data it is conclude that administration of EERDMDK significantly increases anoxia tolerance time, significantly decreases immobility time and number of writhes in animals and this effect was dose dependent. In addition reversal of immobilisation stress induced changes in biochemical parameters and organs weight were also exhibited in EERDMDK treated groups. The possible mechanism underlying this effect is mediated through normalization of catecholamines level. The results of present study are encouraging to pursue further studies on the bioactive guided fractionation of these extract to isolate and characterize probable bioactive molecule responsible for antistress activity.

CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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