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NEUROPROTECTIVE EFFECTS OF *CARISSA CARANDAS* IN EXPERIMENTAL ANIMAL MODEL

PARVEEN K[1*], MARATHA S[2] AND CHAUBEY N[1]

1: Sri Satya Sai University of Technology & Medical Sciences, Sehore, Madhya Pradesh, India-466002

2: Shree Guru Gobind Singh Tricentenary University (SGT University), Budhera, Gurugram, Haryana, India-122505

***Corresponding Author: E Mail:** parveen.punia7798@gmail.com

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ABSTRACT

Carissa carandas (Apocynaceae) a perennial, flowering shrub was evaluated for its neuroprotective effects in various animal models i.e Open Field Activity, Rearing Test, Head Dip Test. CNS Depressant Activity, Traction Test and Cage Crossing Movement. Administration of etahnolic fruit extract extracts of *Carissa carandas*, showed an increase in exploratory functions as compared to the control group in a dose dependant manner. Etahnolic fruit extract extracts of *Carissa carandas* significantly ($p < 0.01$) increase in head-dipping behavior of the animals in Head-dipping tests. A significant ($p<0.01$) dose-dependent antidepressant effect was observed in cage cross activity. A mild dose-dependent muscle-relaxant activity was also observed.

Phytochemical screening of *C. carandas* revealed the presence of alkaloids, glycosides, steroids, saponins, tannins, proteins, phenolic compounds and flavonoids. A number of researcher reported that the alkaloids, glycosides, and flavonoids rich plant extracts possess sedative, anxiolytic, and antiepileptic properties mediated through their affinity with benzodiazepine site of GABAergic complex system in the brain producing drowsiness and facilitating or maintaining sleep.

The results obtained in this study suggest that the *C. carandas* possesses anxiolytic and muscle relaxant properties. Thus, *C. carandas* has potential clinical applications in the management of anxiety and muscle tension disorders. Further investigations are warranted for elucidating the exact mechanism and bioactive compounds.

Keywords: *Carissa carandas*, anxiety, neuroprotective, depression

INTRODUCTION

Carissa carandas is a perennial **[1]** flowering **[2, 3]** and shrub with strong thorns in pairs **[4]** belong to the dogbane family Apocynaceae and found to be widely distributed throughout subtropical and tropical region of Pakistan **[4, 5]**, India **[4,6]**, Bangladesh, Srilanka, Java, Malaysia, Myanmar and Nepal **[4]**. The plant has been cultivated for hedging around home gardens to prevent gar den vegetables from damaging by animals **[5, 6]** and also used as ornamental due to its beautiful cherry-like fruits **[6]**. It is an evergreen plant which is a hardy, drought tolerant plant that thrives well in a wide range of soils. Its common name is Koromcha in Bangladesh and Karanda in English **[7, 8]**.

Recent studies revealed that a number of plant products including polyphenols, terpenes, flavonoids, alkaloids and various plant extracts exert an antioxidant action, anti-inflammation, anti-malaria and others and their use in CNS related disorders **[9]**. Synthetic psychoactive drugs are widely used in the management of central nervous system (CNS) related disorders **[10]**. However, their nonstop and aimless use has prompted adverse effects influencing the endocrine, autonomic, hypersensitive, hematopoietic, and neurological frameworks of the human body **[11]**. Therefore, in the present study *Carissa carandas* was evaluated as potential new remedial drugs with least reactions and maximum potency, which is accepted to be protected and financially viable.

PLANT MATERIAL

The Fresh fruits of *Carissa carandas* Linn. Plant were collected, identified by local farmers. **PREPARATION OF EXTRACT**

Fruits collected and dried for a few days in shed. Dried fruits of *Carissa carandas* Linn. were severed in small pieces and extracted with ethanol after packing into the 'thimble' of the soxhlet apparatus. The extraction phase was set for 8 hours a day with a highest extraction period of 72 hours. Prepared ethanolic extract of *Carissa carandas* Linn. (**EECC**) was concentrated through evaporation in a rotary evaporator

and stored in small jars at 4°C in a refrigerator for future analysis.

PRELIMINARY PHYTOCHEMICAL SCREENING

The ethanolic fruit extract of *Carissa carandas* was subjected to preliminary phytochemical screening **[12, 13]**.

ANIMALS USED FOR ASSESSMENT OF NEUROPHARMACOLOGICAL ACTIVITY

Albino mice either sex (25–30 g) were used in the study. The animals were maintained in standard laboratory conditions (25 °C and light/dark cycles of 12/12 h) and fed with standard food and water ad libitum. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (NU/PH/M/COG/12/78).

ASSESSMENT OF NEUROPHARMACOLOGICAL ACTIVITY

Neuropharmacological activity was studied by open field test, traction test, head dip test, rearing test, and swimming induced depression test. All tests were performed in a calm and peaceful environment. For each test, animals were divided into 5 groups. Each group comprised 5 animals.

Group I- Control received 0.9% NaCl

Group II-Standard received diazepam 2mg/kg.

Group III- EECC at the dose of 250 mg/kg dissolved in Normal saline.

Group IV- EECC at the dose of 500 mg/kg dissolved in Normal saline.

Group V- EECC at the dose of 1000 mg/kg dissolved in Normal saline.

All the drugs were administered orally. The observations were made after 30 min of oral dose of the standard and test substance.

Open Field Activity

The open field apparatus designed in the laboratory consists of 76 X 76 cm square area with opaque walls 42 cm high. The floor is divided by lines into 25 equal squares. Mice weighing 25 to 30gm were used as the test animals in this method. Testing was carried out in a quiet room under white light. Animals taken out from their home cages and were placed in the center square of the open field (one at a time). Number of Squares crossed with all four paws was counted for 30 minutes. Activities of control rats and drug treated rats were monitored in a balanced design to avoid order effect **[14-16]**.

Rearing Test

A 1000-mL glass beaker lined with white paper on the bottom was used in this study. Upward movements of mice positioning the body in an erect position in the beaker were counted **[17-19]**.

Head Dip Test

A specially designed square-shaped head dip box having 3 holes in each side was used in this study. The number of head dips by mice through these holes in a specified time (32–34) was counted. The control and drug-treated animals were placed individually in the head dip box and the observations were made for 30 min **[14-16]**.

CNS Depressant Activity

CNS depressant activity was evaluated by the forced swimming test. All mice were first trained for swimming in a bath with dimensions (42 × 19 × 19 cm) as reported previously (34,35). Mice were placed individually for 6 min in a glass tub filled with water at room temperature (25 ± 2 °C) up to a marked level. Mice suddenly start to move their front and hind paws as soon as they are placed in water. The activity time was determined with the help of stop watch out of a total observation time of 6 min. Mice were considered immobile when they ceased struggling and started making the minimum movements necessary to keep afloat. This is the most commonly used method to evaluate depression **[18-20]**.

Traction Test

This observation was made to determine the time taken by the animal to travel on an iron rod of 1 m in length. Mice were first trained to walk on the iron rod. Any increase or decrease in the time taken by the drug-treated animals from that of the control animals to travel the rod describes the sedative or stimulant activity of the drug, respectively **[21–23]**.

Cage Crossing Movement

The test was carried out in a specifically designed instrument having a rectangular shape. Both control and treated mice were placed in the cage and their cage crossing movements were noted over 30 min. This test was performed according to the method described previously **[24-25]**.

STATISTICAL ANALYSIS

The results are presented as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used for comparison tests of significant differences among groups, followed by Dunnett's't' post-test using GraphPad Software, Inc., La Jolla, CA, USA.

PHYTOCHEMICAL SCREENING

Preliminary phytochemical analysis of EECC. Phytochemical analysis of EECC revealed the presence of phytoconstiruents such as such as alkaloids, carbohydrate, sterols, tannins, phenols, flavanoids, glycoside and saponins **Table 1**.

Open Field Activity

The mean value in the control animals was 311.2 ±0.17.The mean values for EECC at the dose of 100mg/kg, 250 mg/kg and 500 mg/kg were 284±0.42, 265.4±0.41 and 213±0.02. The EECC managed to decrease the no of counts significantly ($p<0.01$) in a dose dependant manner as compared to control (**Table 2**). The mean value (37±0.17) in diazepam treated group were significantly lower as compared with control ($p<0.01$).

Rearing Test

The exploratory rearing activity observed for the control group was 50.8±1.2. The mean values for EECC at the dose of 100mg/kg, 250 mg/kg and 500 mg/kg were 34±1.14, 24.2±0.86 and 21.4±0.9. The EECC managed to decrease the no of rearing significantly ($p<0.01$) in a dose dependant manner as compared to control (**Table 3**). The mean value (10.8±0.86) in diazepam treated group were significantly lower as compared with control ($p<0.01$).

Head Dip Test

The mean value of head dip in the control animals was 62.4±2.5. The mean values for EECC at the dose of 100mg/kg, 250 mg/kg and 500 mg/kg were 53.8±2.7, 76.6±2.6 and 123.6±1.6. The EECC managed to increase the no of head dip significantly ($p<0.01$) in a dose dependant manner as compared to control except at the dose of 100mg/kg which didn't show any significant activity (**Table 4**). The mean value (152.8±2.4) in diazepam treated group were significantly higher as compared with control ($p<0.01$). Increase in no of head dips is a sign of EECC possessing anxiolytic activity.

CNS Depressant Activity

In forced swim test the immobility time was recorded. EECC at the dose of 100mg/kg, 250 mg/kg and 500 mg/kg increase immobility time (2.62±0.12, 2.76±0.20 and 3.32±0.27). The EECC managed to increase the immobility time significantly ($p<0.01$) in a dose dependant manner as compared to control (1.54±0.15) (**Table 5**). The mean value (3.98±0.14) in diazepam treated group were significantly higher as compared with control ($p<0.01$). Increases in immobility times in this test indicate a decrease in swimming and struggling. This shows that EECC has sedative diazepam like action.

Traction Test

The results of motor coordination activity in a traction test were noted for 30 min. The time for crossing the rod was observed and compared with the control and standard drugs. The mean value in the control animals was 8.16±0.08. The mean values for EECC at the dose of 100mg/kg,

250 mg/kg and 500 mg/kg were 9.24±0.18, 10.54±0.77 and 11.66±0.39. The EECC managed to decrease the no of counts significantly ($p<0.01$) in a dose dependant manner as compared to control except at the dose of 100mg/kg which didn't show any significant activity (**Table 6**). The mean value (13.66±0.67) in diazepam treated group were significantly lower as compared with control ($p<0.01$).

Cage Crossing Movement

In the cage cross, the activity observed for the control animals was 87.8±2.3. The mean values for EECC at the dose of 100mg/kg, 250 mg/kg and 500 mg/kg were 68±1.14, 60±1.14 and 60±01.41. The EECC managed to decrease the no of counts significantly ($p<0.01$) in a dose dependant manner as compared to control (**Table 7**). The mean value (10±0.70) in diazepam treated group were significantly lower as compared with control ($p<0.01$).

Table 1: Phytochemical screening of EECC

Chemical constituent	EECC
Carbohydrates	+
Alkaloids	+
Flavonoid	+
Tannins and phenolic	+
Steroids	+
Terpenoids	+

-: negative indicates the absence of the corresponding constituent, +: positive indicates the presence of the corresponding constituent

Table 2: Effect of EECC on locomoter activity in open field test

Group n=5	Treatment	No of counts (mean±SEM)
I	Control (0.9% w/v Nacl)	311.2 ±0.17
II	Standard (Diazepam-2 mg/kg)	37±0.17**
III	EECC(100 mg/kg)	284±0.42**
IV	EECC (300 mg/kg)	265.4±0.41**
V	EECC (500 mg/kg)	213±0.02**

Group I- Control (received 0.9 % w/v NaCl), Group II- Standard (recived Diazepam 2mg/kg), Group III-V-ethanolic extract with different doses; one-way ANOVA followed byValues are given as mean±S.E.M. from five mice in each group, * p < 0.05 significant from control animals, ** p < 0.01 significant from control animals.

Table 3: Effect of EECC on rearing

Group n=5	Treatment	No of Rearing (mean±SEM)
I	Control (0.9% w/v Nacl)	50.8±1.2
II	Standard (Diazepam-2 mg/kg)	10.8±0.86**
III	EECC(100 mg/kg)	34±1.14**
IV	EECC (300 mg/kg)	24.2±0.86**
V	EECC (500 mg/kg)	21.4±0.9**

Group I- Control (received 0.9 % w/v NaCl), Group II- Standard (received Diazepam 2mg/kg), Group III-V-ethanolic extract with different doses; one-way ANOVA followed by Values are given as mean±S.E.M. from five mice in each group, * p < 0.05 significant from control animals, ** p < 0.01 significant from control animals.

Table 4: Effect of EECC in Head dip test

Group n=5	Treatment	No of head dip (mean±SEM)
I	Control (0.9% w/v Nacl)	62.4±2.5
II	Standard (Diazepam-2 mg/kg)	10.8±0.86**
III	EECC(100 mg/kg)	53.8±2.7
IV	EECC (300 mg/kg)	76.6±2.6 **
V	EECC (500 mg/kg)	123.6±1.6**

Group I- Control (received 0.9 % w/v NaCl), Group II- Standard (recived Diazepam 2mg/kg), Group III-V-ethanolic extract with different doses; one-way ANOVA followed byValues are given as mean±S.E.M. from five mice in each group, * p < 0.05 significant from control animals, ** p < 0.01 significant from control animals.

Table 5: Effect of EECC in forced swim test

Group n=5	Treatment	Time(minutes)
I	Control (0.9% w/v Nacl)	1.54±0.15
II	Standard (Diazepam-2 mg/kg)	3.98±0.14**
III	EECC(100 mg/kg)	2.62±0.12**
IV	EECC (300 mg/kg)	2.76±0.20 **
V	EECC (500 mg/kg)	3.32±0.27**

Group I- Control (received 0.9 % w/v NaCl), Group II- Standard (recived Diazepam 2mg/kg), Group III-V-ethanolic extract with different doses; one-way ANOVA followed byValues are given as mean±S.E.M. from five mice in each group, * p < 0.05 significant from control animals, ** p < 0.01 significant from control animals.

Table 6: Effect of EECC in Traction test

Group n=5	Treatment	Time(minutes)
I	Control (0.9% w/v Nacl)	8.16±0.08
II	Standard (Diazepam-2 mg/kg)	13.66±0.67**
III	EECC(100 mg/kg)	9.24±0.18
IV	EECC (300 mg/kg)	10.54±0.77 **
V	EECC (500 mg/kg)	11.66±0.39**

Group I- Control (received 0.9 % w/v NaCl), Group II- Standard (recived Diazepam 2mg/kg), Group III-V-ethanolic extract with different doses; one-way ANOVA followed byValues are given as mean±S.E.M. from five mice in each group, * p < 0.05 significant from control animals, ** p < 0.01 significant from control animals.

Table 7: Effect of EECC in Cage crossing movement

Group n=5	Treatment	Time(minutes)
I	Control (0.9% w/v Nacl)	87.8±2.3
II	Standard (Diazepam-2 mg/kg)	10.0±0.70
III	EECC(100 mg/kg)	68.0±1.14
IV	EECC (300 mg/kg)	60.0±1.41
V	EECC (500 mg/kg)	48.6±1.20

Group I- Control (received 0.9 % w/v NaCl), Group II- Standard (recived Diazepam 2mg/kg), Group III-V-ethanolic extract with different doses; one-way ANOVA followed byValues are given as mean±S.E.M. from five mice in each group, * p < 0.05 significant from control animals, ** p < 0.01 significant from control animals.

DISCUSSION AND CONCLUSION

Administration of etahnolic fruit extract extracts of *C. carandas*, showed an increase in exploratory functions as compared to the control group. Etahnolic fruit extract extracts of *C. carandas* worked in a dose dependant manner. Locomotor activity is considered as an index of alertness and a decrease in it indicates a sedative effect.

Head-dipping behavior of the animals is directly related to their emotional state

[26]. Based on this observation, it was suggested that the expression of an anxiolytic state in animals might be reflected by an increase in head-dipping behavior **[26]** while a decrease in the number of head dips was found to be correlated with the depressant effect **[27-28]**. Likewise, our results demonstrated that etahnolic fruit extract extracts of CC significantly ($p < 0.01$) increase in head-dipping behavior of the animals in Head-dipping tests.

These results taking together indicate that, in contrast to diazepam, etahnolic fruit extract extracts of *C. carandas* showed anxiolytic-like effects without affecting locomotor activity or without producing central nervous depression.

In cage cross activity there was a dose-dependent stimulatory effect, while in rearing the activity was lower at the minimum dose but comparable to s at a maximum dose of 500 mg/kg, such that no sedative effect was observed. In the cage cross and rearing tests the activity was slightly standard reduced at 100 and 300 mg/kg but normal at 500 mg/kg. There was a slight calming effect in cage cross and rearing tests with an increase in dose.

The forced swimming test is frequently used for the assessment of antidepressant-like activity in animal models. The shortening of immobility duration indicates antidepressant activity in this model, while prolonged immobility duration reflects a CNS depression-like effect **[29]**. A significant ($p<0.01$) dose-dependent antidepressant effect was observed.

The traction test was performed in animals for determining the muscle-relaxant potency of the treatments **[30]**. Results from this study revealed that etahnolic fruit extract extracts of *C. carandas* had mild muscle-relaxant activity. This mild effect was observed to be dose-dependent.

A number of researcher reported that the alkaloids, glycosides, and flavonoids rich plant extracts possess sedative, anxiolytic, and antiepileptic properties mediated through their affinity with benzodiazepine site of GABAergic complex system or are direct or indirect modulators of this receptor's increases in GABA activity in the brain producing drowsiness and facilitating or maintaining sleep **[31-35]**. Researchers concluded that the sedative and muscle-relaxant-like properties of benzodiazepines such as diazepam are mostly due to interference with the action of gamma aminobutyric acid (GABA) **[36]**.

Investigations on the phytochemical screening of *C. carandas* revealed the presence of alkaloids, glycosides, steroids,

saponins, tannins, proteins, phenolic compounds and flavonoids. It is possible that the mechanism of anxiolytic action of *C. carandas* could be mediated by synergistic action of these phytochemicals. The results obtained in this study suggest that the *C. carandas* possesses anxiolytic and muscle relaxant properties. Thus, *C. carandas* has potential clinical applications in the management of anxiety and muscle tension disorders. Further investigations are warranted for elucidating the exact mechanism and bioactive compounds.

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