

Research Article**Evaluation of anti-anxiety activity of *Cocos nucifera* endocarp on anxiety models****Virupanagouda P. Patil¹, Nanjappaiah Hankuntimath¹, Chandrashekhar Venkaraddi Mangannavar², Shivakumar Hugar^{1*}**¹P.G. Department of Pharmacology, BLDEA's SSM College of Pharmacy and Research Centre, Vijayapur-586103. Karnataka. India.²P.G. Department of Pharmacology, BVVS's HSK College of Pharmacy, Bagalkot- 587101. Karnataka. India.

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Abstract

Objective: Current study deals with the anti-anxiety effect of ethanolic extract of *Cocos nucifera* endocarp in anxiety models. **Materials and Methods:** Anxiolytic effect of *C. nucifera* endocarp at the dose of 125, 250 and 500 mg/kg evaluated using validated models such as elevated plus maze test, open field apparatus test and light/dark exploration test in mice. **Results:** In the EPM, test extract at 250 and 500 mg/kg significantly increased the number of entries and time spent in open arms. In the OFT, *C. nucifera* endocarp extract significantly increases, the rearings and square crossings. In the LDT, title plant significantly increased the time spent in light chamber, number of crossings and duration of immobility was reduced. **Conclusion:** From our study, it can be concluded that ethanolic extract of *Cocos nucifera* endocarp at the dose of 250 and 500 mg/kg possesses marked anti-anxiety effect.

Keywords: *Cocos nucifera*, anti-anxiety, elevated plus maze test

Introduction

Anxiety has become the integral part in the present era of busy life. Benzodiazepines are commonly used drugs for the management of anxiety. But these drugs possess adverse effects and dependence liability. Hence, there is need of drug from herbal source for the treatment of anxiety, since the side effects and dependence are very less with herbal drugs (Emamghoreishi et al., 2001). Many plants were investigated for their anti-anxiety property (Spinella, 2001; Kamal and Jawaid, 2011). In view of this, plants are traditionally used for the management of neurological disorders like anxiety or anxiety related disorders.

Cocos nucifera L., commonly used in diet, is one of the promising herbs for the treatment of neurological diseases. Previous study indicates that the fruit of *C. nucifera* has been found to be effective therapeutically in the treatment of many ailments like Cardiovascular diseases, metabolic disorders

(Bankar et al., 2011) etc. *C. nucifera* possesses many health beneficial effects like antitumour, bactericidal, aphrodisiac, diuretic, antioxidant, vasorelaxant and antihypertensive (Lima et al., 2015). This study was intended to unearth the scientific evidence on the valuable effects of traditional practices. Hence, we have undertaken present research aimed to investigate the ethanolic extract of *Cocos nucifera* endocarp for the anti-anxiety potential in experimental animal models.

Materials and Methods**Plant material**

Fresh *Cocos nucifera* fruits were collected from local market after authenticated by Dr. M. B. Mulimani, Professor of Botany, S.B Arts and K.C.P. Science College, Bijapur, Karnataka. Its hardest part i.e. endocarp was collected. A voucher specimen (CN02) has been deposited at the herbarium of Dept. of Pharmacology, HSK College of Pharmacy, Bagalkot.

Preparation of extract

Fresh endocarp was grounded using grinder into coarse powder. This powdered material was packed into Soxhlet column and extracted with ethanol (90%). Extract thus obtained was concentrated using rotary flash evaporator.

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Preliminary phytochemical screening

Crude extract was screened to identify the occurrence of various phytoconstituents like polyphenols, flavonoids, alkaloids, glycosides, tannins, saponins, terpenoids etc (Siddiqui et al., 2007).

Total phenolic content (TPC)

The TPC of *Cocos nucifera* extract was estimated by UV spectrophotometry using folin-ciocalteu method. 0.1 ml of the extract was added to reaction tube containing 0.5 ml of folin-ciocalteu reagent (reference). To this add distilled water to make up to 3 ml. 2 ml of sodium carbonate (20%) was added to this reaction mixture after 3 min, and mixed thoroughly. This solution was then incubated at 50°C for 5 min and then cooled. The absorbance of solution was measured at 650 nm against the blank. The TPC of the extract was expressed as mg quercetin equivalent per gm of extract. The coefficient was determined as given below:

$$r^2 = 0.9968.$$

Total flavonoid content (TFC)

Aluminium chloride method was used for the determination of TFC of *Cocos nucifera* extract. 0.5 ml of sample was mixed with 0.5 ml of AlCl₃ ethanol solution (2 %). This solution was incubated at room temperature for 01 hour. The absorbance of this mixture was measured at 420 nm. The TFC was calculated as mg quercetin equivalent per gm of extract. The coefficient of determination was $r^2 = 0.9965$ (Krishnamoorthy et al., 2011).

Determination of acute toxicity (LD₅₀)

This investigation was carried out as per the guidelines approved by the Institutional Animal Ethical Committee (IAEC: BPC/82/2014). In this research, the mice were fasted for 4 hr before the experiment. After dosing, food was withheld for further 1 hr and water *ad libitum*. General behaviour and mortality of the mice observed individually daily for a total of 14 days. Fixed dose method of OECD Guideline No. 423; (Annexure-2d: Starting dose: 2000 mg/kg b.w) was followed for toxicity study (Veeraraghavan, 2001).

Evaluation of anti-anxiety activity of *Cocos nucifera* endocarp

Experimental design

Albino mice (20-30 g) of either sex were divided into 05 groups and each group contains 06 mice fasted overnight prior to the test but water was supplied *ad libitum*.

Group I - Normal control.

Group II - Diazepam (1 mg/kg, p.o.)

Group III - EECNE (Ethanol extract of *Cocos nucifera* endocarp) 125 mg/kg, p.o.

Group IV - EECNE 250 mg/kg, p.o.

Group V - EECNE 500 mg/kg, p.o.

Elevated plus maze (EPM)

This EPM is the most widely used apparatus to investigate effects of drugs on CNS. This apparatus consisting of four arms: two open arms (35 x 6 cm) and two closed arms (35 x 6 x 15 cm). These arms were extended from a central platform and were elevated to a height of 45 cm above the floor.

All the groups received respective treatment once daily for 10 days. On the last day one hour after the treatment, each mouse was placed individually on the center of the elevated plus maze, head facing towards open arm. Number of entries and time spent in the open arm parameters were recorded for 5 min.

Open field apparatus test (OFT)

The treatment was given once daily for 7 days. On 7th day 60 min after administration each mouse was placed in the center of open field and the parameters such as ambulation (measured in terms of the number of squares crossed by the animal) and rearing (number of times, the animal stood on its hind limbs) were recorded during a test period of 5 min (Hemant et al., 2016).

Light-dark test (LDT)

The LDT is the commonly used model to assess anti-anxiety efficacy of drugs. This apparatus consists an acrylic box (40 cm x 60 cm x 20 cm) divided into light (40 cm x 40 cm) and dark chambers (40 cm x 20 cm). The light chamber (painted white color) connected via an opening (7 cm) at floor level to the dark chamber (painted black). A white light lamp (60 W) was placed approximately 40 cm above from the light chamber.

The treatment was continued once daily for 7 days. On 7th day 60 min after administration each mouse was placed in the light chamber, which faces the opening into the dark chamber. Observations recorded were time spent in the light compartment, number of squares crossed and duration of Immobility during 5 min of test (Bourin and Hascoet, 2003).

Statistical analysis

Data expressed as mean \pm standard error mean (SEM). The data thus obtained were subjected to statistical analysis of ANOVA test followed by Tukey's Kramer Multiple Comparison Test to assess the statistical significance of the results. Statistical analysis was done using GraphPad Prism-5 software. P-values < 0.05 were considered as statistically significant.

Results

Preliminary phytochemical screening

It was found that, the ethanolic extract of *Cocos nucifera* contains polyphenols, flavonoids, alkaloids, carbohydrates, proteins and diterpenes.

Total phenolics and flavonoids contents

The TPC of extract was determined by the Folin-Ciocalteu reagent. The phenolic content was found to be 48.37 ± 2.61 mg quercetin/g. The TFC was evaluated using a colorimetric assay method and it was found to be 39.72 ± 2.85 mg quercetin/g.

Acute toxicity study

Based on the results obtained, 3 graded screening doses like $1/5^{\text{th}}$ (500 mg/kg), $1/10^{\text{th}}$ (250 mg/kg) and $1/20^{\text{th}}$ (125 mg/kg) of LD_{50} cut off value were selected for exploration of anti-anxiety activity of EECNE.

Effect of *Cocos nucifera* extract on the elevated plus-maze

Results are presented in table 1. During 5 min test, control animals spent more time in closed arm and showed less entries in

open arm compared to closed arm. Whereas diazepam treated animals showed significant increase in the % of open arms entries as well as time spent in open arm. Ethanolic extract of *Cocos nucifera* (at 250 and 500 mg/kg) also exhibited significant increase in the % of number of open arm entries and time spent in open arm. However, 125 mg/kg has also exhibited increase in the % of entries into open arms and time spent in open arm but results were found to be statistically non-significant.

Effect of *Cocos nucifera* extract on open field test

In this test, standard and extract (*C. nucifera* at 250 and 500 mg/kg) treated mice showed significant increase in the number of rearings and squares crossed during 5-min interval as compared to normal control group. Whereas, the test extract at 125 mg/kg p.o. showed statistically non-significant results. All the data tabulated in table 2.

Effects of *Cocos nucifera* extract on Light dark test

Standard drug, diazepam significantly increased the time spent in light box and the number of crossings between the

Table 1. Effect of *Cocos nucifera* extract on the elevated plus-maze behaviour in mice

Treatment	Dose	% Entry into open arm	% time spent in open arm
Control	---	42.47±2.51	37.93±2.07
Std (Diazepam)	1 mg/kg p.o.	78.29±5.32***	74.77±4.29***
EECNE	125 mg/kg p.o.	46.81±3.28 ^{ns}	43.67±3.88 ^{ns}
EECNE	250 mg/kg p.o.	64.53±5.40*	63.39±4.16**
EECNE	500 mg/kg p.o.	74.77±6.86**	69.90±4.37***

Values are Mean SEM, (n=6), ^{ns}Non-significant, *P<0.05, **P<0.01, ***P<0.001 as compared to control.

Table 2. Effect of *Cocos nucifera* extract on the behaviour of animals in open field test

Treatment	Dose	No. of squares crossed	Rearing
Control	---	122.47±2.51	10.38±1.49
Std (Diazepam)	1 mg/kg p.o.	178.29±5.32***	28.82±2.64***
EECNE	125 mg/kg p.o.	131.72±4.63 ^{ns}	13.45±2.47 ^{ns}
EECNE	250 mg/kg p.o.	152.56±4.91**	19.88±3.26*
EECNE	500 mg/kg p.o.	172.97±5.75***	27.40±3.05**

Values are Mean SEM, (n=6), ^{ns}Non-significant, *P<0.05, **P<0.01, ***P<0.001 as compared to control.

Table 3. Effects of *Cocos nucifera* extract on the light dark test behaviour in mice.

Treatment	Dose	Time spent in Lighted box	No. of squares crossed	Duration of Immobility
Control	---	97.49±11.72	10.90±1.64	38.48±2.21
Std (Diazepam)	1 mg/kg p.o.	188.20±14.41***	31.03±2.25***	18.98±2.08***
EECNE	125 mg/kg p.o.	146.44±12.99 ^{ns}	16.61±2.74 ^{ns}	36.18±3.35*
EECNE	250 mg/kg p.o.	162.46±12.02**	25.07±2.36**	26.47±2.51**
EECNE	500 mg/kg p.o.	185.28±15.77***	29.82±3.57***	20.06±2.72***

Values are Mean SEM, (n=6), ^{ns}Non-significant, *P<0.05, **P<0.01, ***P<0.001 as compared to control.

light and dark boxes, but duration of immobility was significantly reduced. EECNE treated mice also exhibited dose dependent (250 and 500 mg) significant increase in the time spent in light box as well as the number of crossings between light and dark boxes. The duration of immobility was also significantly reduced by EECNE, in a dose dependent manner as compared to the control group.

Discussion

Our investigation results indicates that EECNE administered mice exhibited a significant anxiolytic effect in all the anxiety models. In the present study, administration of EECNE produced an increased time spent by animal in the illuminated side on the light dark test indicates an anti-anxiety potential of *Cocos nucifera*, which was further evident by the increased time spent in the open arms on the elevated plus maze.

The EPM test is one of the most commonly employed model to determine the anti-anxiety agents. This model employ natural stimuli i.e. fear of open space and fear of balancing on narrow raised arms. It is well-known that anti-anxiety drugs certainly increase the number of entries and time spent in the open arms. Our study demonstrates the anti-anxiety efficacy, since significant decrease in anxiety like behaviour by increasing the time spent in open arm (Pitchaiah et al., 2013).

The open field apparatus test is used to measure the animal emotional condition. This model produces anxiety-related behaviour, which is characterized by animal's aversion to an open and bright area. Thus, when animals placed in the OFT chamber they show fear or anxiety behaviour. Anti-anxiety drugs decrease such fearful behaviour of animals in OFT (Mechan et al., 2002). *Cocos nucifera* extract exhibits anxiolytic effect as there is significant increase in the parameters like number of rearings and number of squares crossed.

LDT model is an ethologically based model of fear and anxiety. This model examines the number of animal entries between the light and dark compartments and the time spent in the light compartment as anxiety indices (Shalini and Neeraj, 2015). Animals pre-treated with ethanol extract of *Cocos nucifera* exhibited increase in the time spent of animals in the light compartment, confirming the anxiolytic effect of extract. This observed anti-anxiety potential of the *Cocos nucifera* could be due to the agonistic effect on GABA/benzodiazepine receptor complex, or 5-HT1A receptor (Pooja et al., 2016; Millan et al., 1997).

Literature reports on the phytoconstituents and their pharmacology indicates that the plants containing flavonoids, phenolic compounds, alkaloids and tannins exhibits activity against many disorders of CNS (Bhattacharya and Satyan, 1997). It is possible that the mechanism of anxiolytic action of *Cocos nucifera* endocarp may be due to presence of phytochemicals like flavonoids or phenolic compounds.

Conclusion

Results of this investigation exhibits the anti-anxiety effect of *Cocos nucifera* endocarp in animal models and this observed effect could be due to the presence of phytoconstituents.

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Author's contributions

Conception and design of the work were done by Mr. Virupanagouda P. Patil, Dr. Nanjappaiah H. M. and Dr. Shivakumar Hugar. Statistical analysis, interpretation of data and drafting of the article were done by Mr. Virupanagouda P. Patil, Dr. Hugar Shivakumar and Dr. Chandrashekhar V. M.

Conflict of interest

Authors declare that there is no conflict of interest.

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