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ANXIOLYTIC EFFECT OF ETHANOLIC EXTRACT OF AERIAL PARTS OF *CARISSA CARANDAS* L. IN MICE

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ABSTRACT

Medicinal properties of *Carissa carandas* L. are well documented. Its central nervous system related activities are mentioned in Ayurveda system of medicine. However, studies evaluating its anxiolytic properties and the underlying mechanisms are lacking. We investigate anxiolytic activity of Ethanolic extract of aerial parts *Carissa carandas* L. by using animal models such as Elevated plus maze and light dark model. The level of neurotransmitters such as GABA and serotonin in subcortical region of brain of mice were also estimated. The phytoconstituents were extracted using Soxhlet apparatus. The mice were orally administered with the standard drug diazepam for 21 days. Anxiolytic activity was assessed using elevated plus maze and light/dark transition test models on day 1, 7, 14 and 21. On the 21th day, subcortical regions of brain were quantitatively assessed for neurotransmitters. Extract treatment improved exploratory activity in the animal models of anxiety, and also showed increased GABA and

serotonin levels in subcortical regions of brain. Thus, our study concludes that the ethanolic extract of aerial parts *Carissa carandas* shows anxiolytic activity.

Keywords: *Carissa carandas*, elevated plus maze model, Light dark model, Anxiety, GABA, Serotonin

1. INTRODUCTION: -

Anxiety is characterized by cognitive, emotional, and behavioural elements as well as an uneasy sensation linked to nervousness, trepidation, or concern. It affects a person's cognitive functions, including thinking, decision-making, learning, memory, and concentration **[1]**.

A plenty of drug classes provide anxiolytic effects. Benzodiazepines, beta-adrenergic antagonists (propranolol), azapirones such as buspirone, SSRIs and SNRIs are the main treatments for anxiety-related illnesses. However, long-term use of benzodiazepines leads to pharmacological dependency as well as physical dependence, including drowsiness, myorelaxation, ataxia, forgetfulness, and sleepiness. Buspirone also has side effects such as diarrhea, excitement, perspiration, paranesthesia, headache, dizziness, and nervousness **[2]**. While many synthetic pharmaceuticals have a variety of adverse effects, herbal medicines provide safe and well-tolerated treatments **[3]**. From ancient times many medicinal plants were used around the world for the management of anxiety as they do not cause any side effect.

Apocyanaceae family member *Carissa carandas* L., also known as karvanda. It is a woody, climbing shrub that typically reaches heights of 10 to 15 feet (3-5 metres). The plant has historically been used as an antipyretic, an astringent and for conditions affecting the brain **[4]**. Numerous activities, including anticonvulsant, anticancer, histamine-releasing, cardiotonic, neuropharmacological, diuretic, anti-diabetic, antipyretic, hepatoprotective, antihyperlipidemic, and anti-inflammatory, have been linked to the various sections of the *Carissa carandas* plant **[5]**.

The *Carissa carandas* possesses various chemical constituents such as flavonoids, steroids, carbohydrates, alkaloids, triterpenoids and volatile oils. The chemical analysis of stem shows that it contains sesquiterpene glucoside. The chemical analysis of leaves shows that it contains triterpenoids and tannins such as carissic acid, ursolic acid triterpene carandinol, betulinic acid, β-sitosterol-3-O-βd-glucopyranoside, oleanolic acid, ursolic acid, and 4-hydroxybenzoic acid. Fruits of *C. carandas* have been reported to contain carissol, epimer of α-amyrin, linalool, β-caryophyllene, carissone, carissic acid, carindone, ursolic acid, carinol, ascorbic acid, lupeol β-sitosterol, rutin, epicatechin, quercetin, kaempferol, p-coumaric acid,

caffeic acid, ellagic acid **[5, 6, 12]**. The Phytochemicals such as β-caryophyllene [9], rutin, β-sitosterol **[10]**, α-amyrin **[8]**, ursolic acid **[7]**, linalool **[11]** possess anxiolytic properties. The present study was therefore aimed to evaluate the antianxiety activity of *C. carandas* and its underlying mechanism of action. The animal behaviour was evaluated using validated animal models such as elevated plus maze and light and dark paradigm. Further, neurotransmitter levels were quantitatively estimated in subcortical region of the brain.

2. MATERIALS AND METHODS: -

2.1 Preparation of drug solution: -

Diazepam was procured as a gift sample from Research-lab Fine Chem. GABA and Serotonin were procured from Ozone international Mumbai and Himedia, pvt. Ltd. Respectively. Diazepam (1mg/kg) was prepared in 1% Gum acacia solution.

2.2 Collection and authentication of plant material: -

The aerial parts of *Carissa Carandas* L. such as Leaves, stem, Flowers, fruits were collected during the month of February and March 2021. Authentication of plant was done at Kasturbai Walchand College, Sangli, by Dr. Wadhmare Sir, HOD of Botany department.

2.3 Preparation of extract: -

Every aerial component (leaf, stem, flower, and fruit) was cleaned, dried in the shade, and ground into a coarse powder. The resultant dried powder (30 gm) was defatted using petroleum ether maceration. In a Soxhlet apparatus, the powder was extracted using ethanol as the solvent. The obtained ethanol extract was dried and concentrated at room temperature. Prior to being used for animal experiments, the test sample of ethanol extract was prepared using distilled water in the proper concentrations.

2.4. Preliminary qualitative phytochemical analysis: -

Different classes of photochemical constituents such as alkaloids, tannins, flavonoids, saponins, cardiac glycosides and triterpenoids were detected by subjecting extract to preliminary qualitative screening.

2.5. Acute Toxicity Study: -

Fixed dose method of OECD Guideline No. 423; (Annexure- 2d: Starting dose is: 2000mg/kg b. w) was followed for toxicity study. Six mice weighing 25–30 g was used in the investigation to determine the acute oral toxicity. Three hours before the experiment, the mice were fasted. A single, high dose (2000 mg/kg) of the *Carissa Carandas* (Aerial parts) extract was given to the mice via oral gavage. Individual animal behavior, including mortality, was recorded after each dose at least once in the first 30 minutes, several times in the first 24 hours, with a focus on the first four hours, and then every day for a total of 14 days. Based on the study's findings, a screening dose of 200 mg/kg (1/10th of the LD50 cut off value)

was chosen for the evaluation of anxiolytic potential **[13]**.

2.6. Procurement of animals and ethics approval: -

Swiss albino mice weighing 25-30 gm of either sex was used in present study. Animal were splited into 4 groups of 6 mice in each group for the duration of 2 weeks. The experiment was carried out after approval and clearance from IAEC constituted in accordance of CPCSEA, India (Protocol no – IAEC/ABCP/09/2022-23) of Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra. (843/PO/Re/S/04/CPCSEA) India.

2.7. Behavioural tests: -

2.7.1. Experimental design: -

To investigate the anxiolytic like effects of plant extract, the animals were divided into four groups (n = 6 per group): control (1 % gum acacia), diazepam (1 mg/kg) treated, EEACC (100, 200 mg/kg) treated. The diazepam was prepared freshly by suspending in 1 % gum acacia solution. The extract and diazepam were orally administered using a gavage needle. The treatment was given for 21 days. The Anxiolytic activity was evaluated on 1st 7th, 14th and 21st day. After 21-day treatment the mice were sacrificed, brain was isolated and level of neurotransmitters were estimated.

2.7.2. Elevated plus maze apparatus: -

The head of each mouse was positioned facing the open arm in the middle of the elevated plus maze. The mice were observed acting in the following ways throughout the five-minute trial.

- The quantity of admissions into and amount of time spent in the open arm.
- The quantity of admissions into and amount of time spent in the closed arm.
- Anxiety Index **[14]**

Formula: -

- $Anxiety\ Index\ =$ $1 - [time\ spent\ in\ open\ arm \div total\ time\ (300sec) + no.of\ entries\ in\ open\ arm \div total\ no.of\ entries\ in\ both\ the\ arms] \div 2$

2.7.3. Light–dark test (LDT): -

The sensitive model frequently used to identify activity in disorders related to anxiety is the light-dark test. The following observations will be manually recorded throughout the course of a 10-min trial with each mouse put in the light room facing the entrance to the dark chamber.

- Time spent in the light chamber
- Time spent in the dark chamber
- Number of transitions [14]

2.8. Biochemical estimation of neurotransmitters from brain: -

2.8.1. Preparation of tissue extracts: -

Mice were sacrificed on the experimentation day, and the sub-cortical region, including the striatum, was isolated from the rest of the brain. Wet tissue was measured and homogenized in 5 ml of HCl-butanol for about a minute. The sample was subsequently centrifuged for 10 minutes at 2000 rpm. 1 ml of the supernatant phase was removed and added to 2.5 ml of heptane and 0.31 ml of 0.1 M HCl in a centrifuge tube. The tube was shaken for 10 minutes, after which the two phases were separated by centrifugation at 2000 rpm for 10 minutes. The top organic phase was then discarded. Then, 0.2 ml of the aqueous phase was collected for 5-HT. At 0°C, all procedures were carried out **[15]**.

2.8.2. Estimation of GABA level: -

By using the multiple development paper chromatography method, the level of GABA was determined.

Assay-procedure: -

The brain homogenate supernatant was evaporated to dryness in an oven at 70°C, and the residue was then reconstituted in 100 ml of distilled water. The sample is spotted on Whatman no. 1 chromatography paper using a micropipette and the standard GABA solution, which has a concentration of 2 M. It was set down on a chamber that held a solvent solution of butanol, acetic acid, and water (12 v/v: 3 v/v: 5 v/v). When the solvent front reached the top of the paper, it was pulled and dried. The steps are the same for the second run. The papers are then dried, coated with ninhydrin reagent, and baked for 4 minutes at 100°C. Using 0.005% CuSo4 in 75% ethanol, the fractions that contain GABA in line with the standard are cut and eluted. In a spectrophotometer, their absorbance was measured at 515 nm against a blank **[15]**.

Calibration Curve –

Prepare standard GABA solutions containing 2.5–60 g/ml in 0.1N 80% ethanol. These dilutions were then employed in paper chromatography. Whatman spotted filter paper that has been spot-cut and submerged in n-Butanol: Acetic acid is eventually measured at 550 nm in a spectrophotometer using water (12:3:5 v/v) as the solvent solution **[16]**.

2.8.3. Estimation of serotonin: -

Assay Reagents: OPT reagent: (20 mg in 100 ml conc. HCl)

Procedure: -

To 0.2 ml of aqueous extract, 0.25 ml of OPT reagent was added. It took 10 minutes of heating to 100°C to produce the fluorophore. Spectrofluorometer readings were made between 360 and 470 nm once the samples had reached thermal equilibrium with the surroundings. 0.25 ml of concentrated HCI was added to the tissue blank without the OPT reagent. Internal standard: HCl and butanol in a 1:2 ratio, produced with 500 g/ml of serotonin in distilled water **[15]**.

2.9 Statistical Analysis: -

The date was stated as mean ± SEM. From the study, the obtained biochemical parameters were statistically analysed using Two-way ANOVA followed by Dunnett's test. The software GraphPad Prism was used for analysis of result.

3. RESULTS: -

3.1. Phytochemical analysis of plant extract: -

Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, triterpenoids, coumarin glycosides, tannins and saponins.

3.2. Acute Toxicity Studies: -

When plant extract was administered orally to animal, changes in body weight were noted. The changes were minor and indicated that the plant extract did not influence the animal growth.

For evaluating the toxicity of the extract of aerial parts of Carissa carandas, various parameters were seen. All the treatment and control animal's fur, mucus membrane, eyes, salivation, behavioural patterns and sleep were observed to be normal. None of the animals shows the sign of lethargy, tremors, diarrhoea or comma, and all of them showed normal life.

3.3. Behavioral activity of ethanolic extract (aerial parts) *Carissa carandas* L. in mice: -

3.3.1. Elevated plus maze test: -

Table 1: Effect of Ethanolic extract of *Carissa carandas* aerial parts on number of entries in open arms

Groups	Day 1	Day 7	Day 14	Day 21
Control	0.33±0.21	1.16±0.16	1.33±0.21	1.66±0.33
Standard-diazepam	2.5±0.22 **	6±0.63 ****	7.16±0.30 ****	8.5±0.34 ****
Extract (100mg/kg)	1.16±0.16 ns	3.83±0.47 ***	4±0.25 ****	5.16±0.94 ****
Extract (200mg/kg)	1.33±0.21 ns	4.16±0.60 ****	4.5±0.22 ****	5.83±0.87 ****

Values are stated in a mean ± SEM and n=6. *p<0.05 is considered as criteria for significance when compared with control group using two-way ANOVA couples with Dunnett's test for comparison. ns is considered as non-significant

Table 2: Effect of Ethanolic extract of *Carissa carandas* aerial parts on number of entries in closed arms

Groups	Day 1	Day 7	Day 14	Day 21
Control	13.66±0.76	13±0.30	13.16±0.47	13.33±0.42
Standard-diazepam	8.33±0.33 ****	6.66±0.42 ****	5.33±0.33 ****	4±0.36 ****
EEACC (100mg/kg)	12±0.258 **	10±0.47 ****	8.66±0.21 ****	7.83±0.30 ****
EEACC (200mg/kg)	11.5±0.67 *	9.66±0.55 ****	7.66±0.49 ****	7±0.36 ****

Values are stated in a mean ± SEM and n=6. *p<0.05 is considered as criteria for significance when compared with control group using two-way ANOVA couples with Dunnett's test for comparison. ns is considered as non-significant

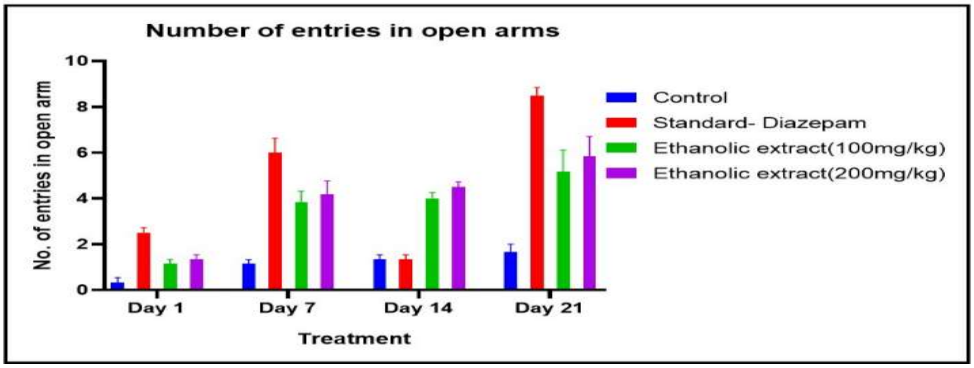

Figure 1: Effect of extract on no. of entries in open arm

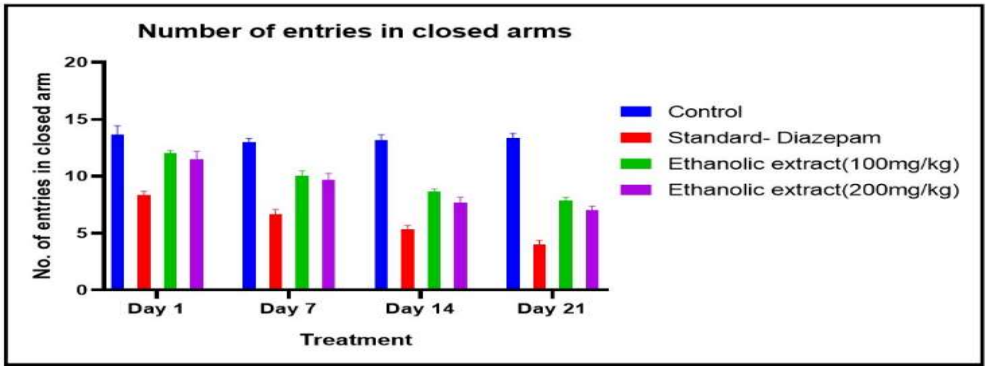

Figure 2: Effect of extract on no. of entries in closed arms

Table 3: Effect of Ethanolic extract of *Carissa carandas* aerial parts on time spent in open arms(s)

Groups	Day 1	Day 7	Day 14	Day 21
Control	2.33±1.49	4.5±0.56	12±1.46	12.16±0.47
Standard-diazepam	31.16±1.85 ****	62.88±1.07 ****	74.66±1.07 ****	81.5±1.38 ****
EEACC (100mg/kg)	8.16±1.30 *	31.16±1.72 ****	40.50±1.52 ****	48.50±1.36 ****
EEACC (200mg/kg)	10.66±1.49 **	40±1.73 ****	53.66±1.11 ****	60.0±1.57 ****

Values are stated in a mean ± SEM and n=6. *p<0.05 is considered as criteria for significance when compared with control group using two-way ANOVA couples with Dunnett's test for comparison. ns is considered as non-significant

Table 4: Effect of Ethanolic extract of *Carissa carandas* aerial parts on time spent in closed arms(s)

Groups	Day 1	Day 7	Day 14	Day 21
Control	196.5±3.43	196.16±1.44	193.66±1.38	183.16±1.62
Standard-diazepam	131.5±1.05 ****	102.83±1.81 ****	97.50±3.14 ****	94.33±2.02 ****
EEACC (100mg/kg)	190.83±1.24 ns	126±0.73 ****	119.33±0.88 ****	110.50±2.40 ****
EEACC (200mg/kg)	186.0±2.06 **	120±0.68 ****	112±1.99 ****	108.33±2.70 ****

Values are stated in a mean ± SEM and n=6. *p<0.05 is considered as criteria for significance when compared with control group using two-way ANOVA couples with Dunnett's test for comparison. ns is considered as non-significant

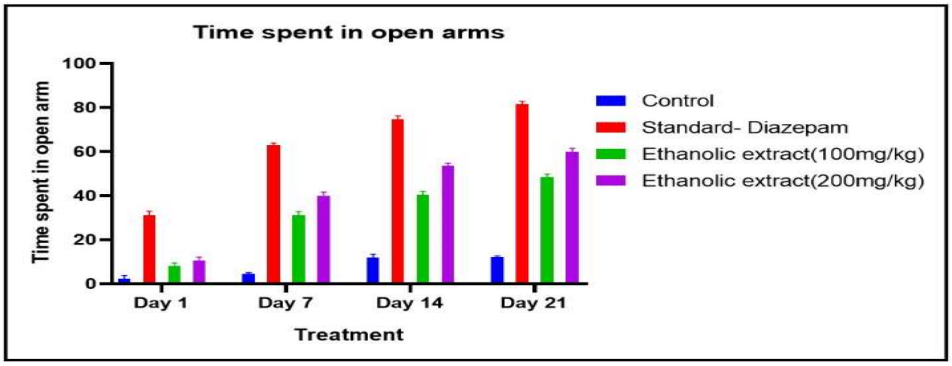

Figure 3: Effect of extract on time spent in open arms

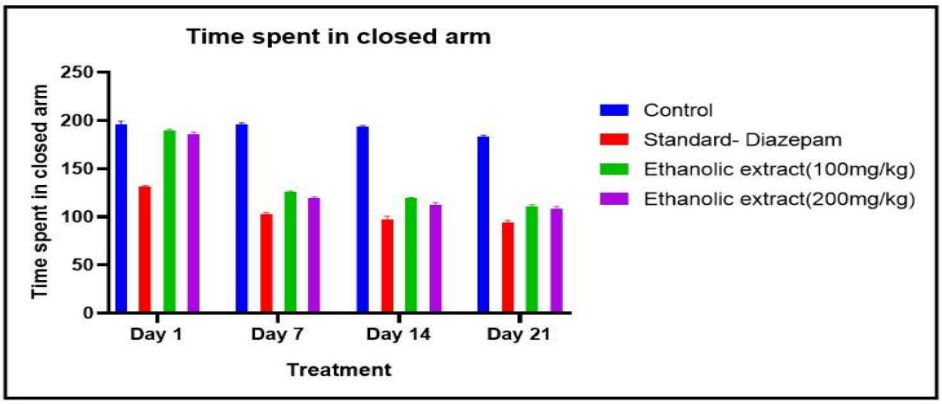

Figure 4: Effect of extract on time spent in closed arms

Anxiety index: -

Table 5: Effect of Ethanolic extract of *Carissa carandas* aerial parts on anxiety index.

Groups	Day 1	Day 7	Day 14	Day 21
Control	0.98	0.98	0.95	0.95
Standard-diazepam	0.30	0.10	0.07	0.02
EEACC (100mg/kg)	0.41	0.22	0.20	0.20
EEACC (200mg/kg)	0.54	0.20	0.10	0.08

Values are stated in a mean ± SEM and n=6. *p<0.05 is considered as criteria for significance when compared with control group using two-way ANOVA couples with Dunnett's test for comparison. ns is considered as non-significant

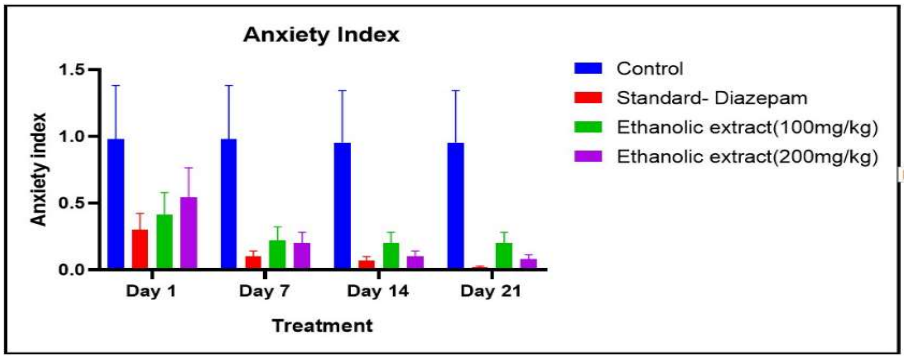

Figure 5: Effect of extract on anxiety index

3.3.2. Light dark Model: -

Time spent in light compartment: -

Table 6: Effect of Ethanolic extract of *Carissa carandas* aerial parts on time spent in light compartment(s)

Groups	Day 1	Day 7	Day 14	Day 21
Control	140.66±2.72	175.66±2.18	178.16±2.31	179.66±1.81
Standard-diazepam	239.16±1.01 ****	277.83±1.88 ****	294.66±1.68 ****	308.33±4.55 ****
EEACC (100mg/kg)	146.00±2.33 ns	228.50±4.49 ****	244.50±2.04 ****	252.50±2.47 ****
EEACC (200mg/kg)	156.50±3.97 ***	248.00±2.92 ****	267.00±2.93 ****	277.00±2.08 ****

Values are stated in a mean ± SEM and n=6. *p<0.05 is considered as criteria for significance when compared with control group using two-way ANOVA couples with Dunnett's test for comparison. ns is considered as non-significant

Time spent in dark compartment: -

Table 7: Effect of Ethanolic extract of *Carissa carandas* aerial parts on time spent in dark compartment(s)

Groups	Day 1	Day 7	Day 14	Day 21
Control	352.83±2.15	332.50±2.81	317.50±3.81	312.50±4.35
Standard-diazepam	303.66±2.36 ****	213.50±3.11 ****	204.33±2.72 ****	180.33±2.52 ****
EEACC (100mg/kg)	343.33±3.56 ns	278.83±2.65 ****	264.66±2.89 ****	232.00±2.30 ****
EEACC (200mg/kg)	335.66±2.96 **	264.00±4.30 ****	247.50±3.99 ****	219.50±5.33 ****

Values are stated in a mean ± SEM and n=6. *p<0.05 is considered as criteria for significance when compared with control group using two-way ANOVA couples with Dunnett's test for comparison. ns is considered as non-significant

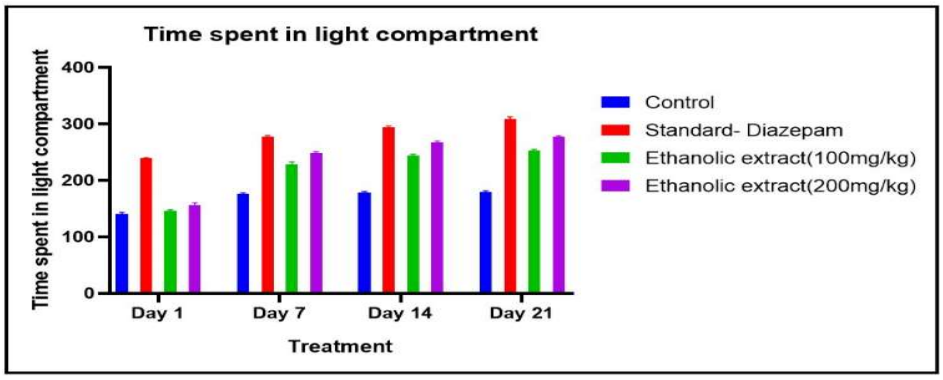

Figure 6: Effect of extract on time spent in light compartment

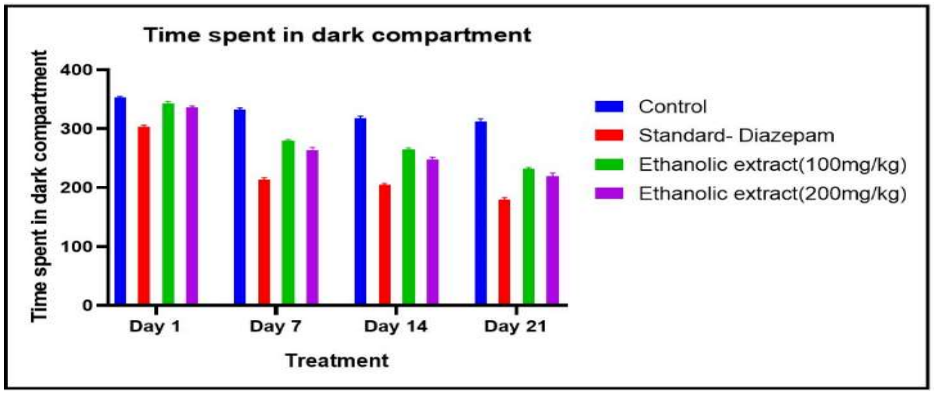

Figure 7: Effect of extract on time spent in dark compartment

Number of Transitions: -

Table 8: Effect of Ethanolic extract of *Carissa carandas* aerial parts on number of transitions

Groups	Day 1	Day 7	Day 14	Day 21
Control	28.5±1.52	28.16±2.32	28.66±1.68	31.00±1.15
Standard-diazepam	43.83±2.62 ****	48.50±3.60 ****	52.50±1.91 ****	52.66±3.14 ****
EEACC (100mg/kg)	32.16±2.24 ns	36.16±1.95 *	40.50±2.78 ***	43.66±1.38 ***
EEACC (200mg/kg)	34.66±2.51 ns	39.66±2.18 **	43.83±1.51 ***	45.16±1.35 ****

Values are stated in a mean ± SEM and n=6. *p<0.05 is considered as criteria for significance when compared with control group using two-way ANOVA couples with Dunnett's test for comparison. ns is considered as non-significant

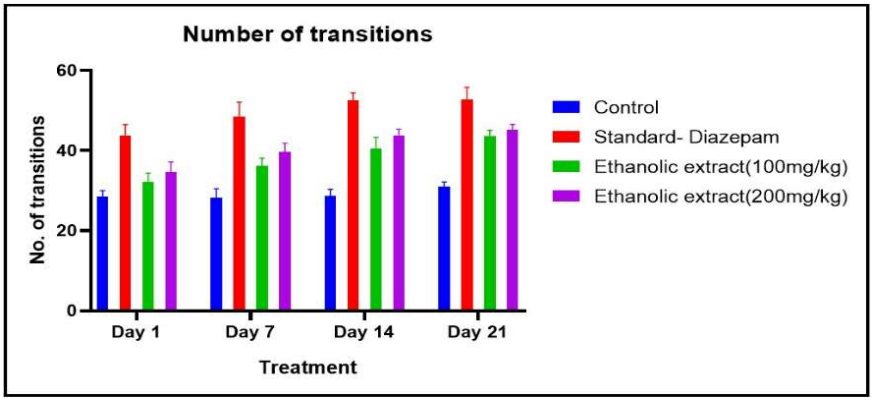

Figure 8: Effect of extract on number of transitions

3.3.3. Neurotransmitter estimation: -

Level of GABA in brain tissue homogenate in mice

Table 9: Effect of extract on level of GABA in brain homogenate of mice.

Groups	Dose, route of administration	GABA (µg/ml) (mean± SEM)
Control	Gum acacia (1%), P.O.	13.54±0.51
Standard-diazepam	1 mg/kg, P.O.	45.50±0.98****
Ethanolic extract	100mg/kg, P.O.	28.28±0.61***
Ethanolic extract	200mg/kg, P.O.	33.43±0.99****

Values are stated in a mean ± SEM and n=6. *p<0.05 is considered as criteria for significance when compared with control group using two-way ANOVA couples with Dunnett's test for comparison. ns is considered as non-significant

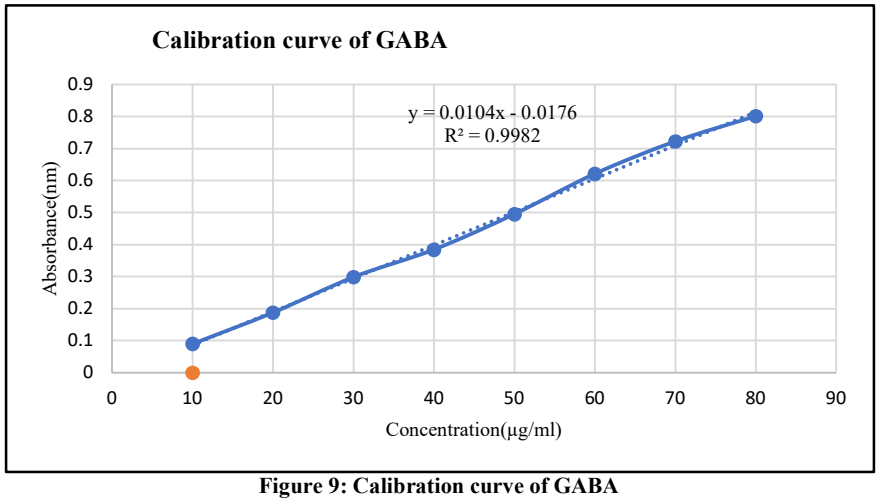

Figure 9: Calibration curve of GABA

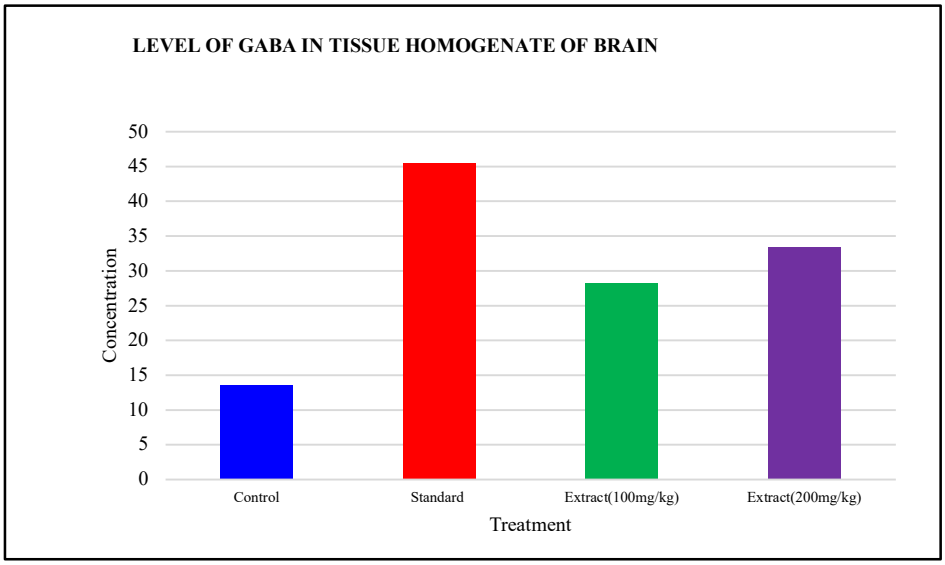

Figure 10: Effect of extract on level of GABA in brain homogenate of mice

Results of serotonin estimation: -

Level of Serotonin in brain tissue homogenate in mice

Table 10: Effect of extract on level of Serotonin in brain homogenate in mice.

Groups	Dose, route of administration	Serotonin (µg/ml) (mean± SEM)
Control	Gum acacia (1%), P.O.	67.29±1.41
Standard-diazepam	1 mg/kg, P.O.	32.10±0.61****
Ethanolic extract	100mg/kg, P.O.	52.73±1.43***
Ethanolic extract	200mg/kg, P.O.	43.02±0.98****

Values are stated in a mean ± SEM and n=6. *p<0.05 is considered as criteria for significance when compared with control group using two-way ANOVA couples with Dunnett's test for comparison. ns is considered as non-significant

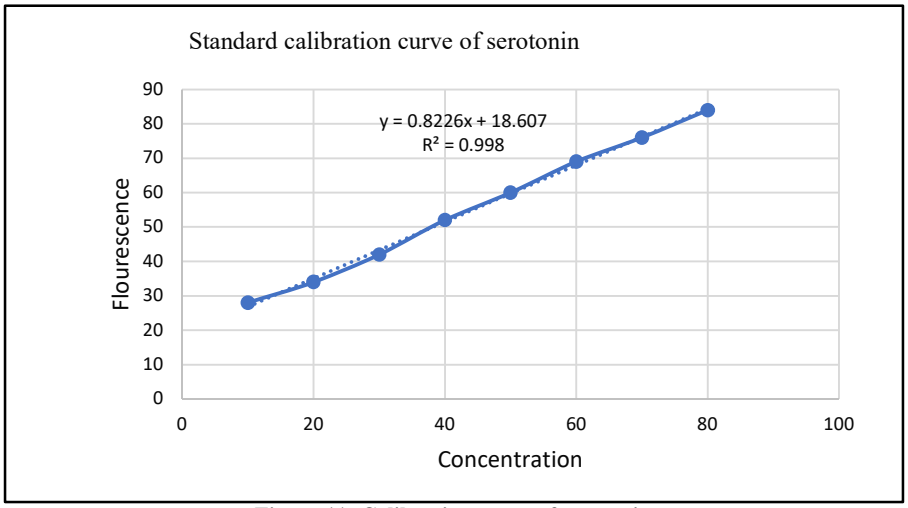

Figure 11: Calibration curve of serotonin

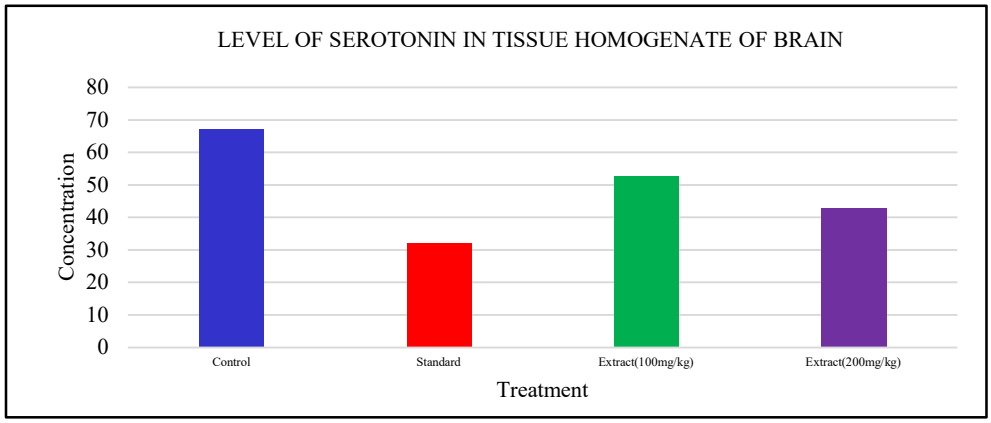

Figure 12: Effect of extract on level of serotonin in brain homogenate of mice

DISCUSSION: -

The effect of ethanol extracts of *Carissa carandas* on anxiety was examined in the current study using EPM and LDT animal models. These two animal models are both predictively valid and have face validity. This indicates the therapeutic efficacy of anxiolytic medicines or the reverse effects of anxiogenic medications **[16]**.

The many parts of the brain that are implicated in anxiety include the hippocampus, amygdala, and thalamus. These parts of the brain were chosen for our investigation because they have been linked to anxiety. GABA and serotonin are neurotransmitters that are implicated in the emergence of anxiety disorders. The key to treating anxiety disorders with pharmacological drugs is to reinstate the function of these neurotransmitter systems, especially GABA and 5-HT **[16]**.

Anxiety disorders are caused by abnormalities in the serotonergic system, including hypo- or hyperactivity of serotonin neurotransmission in important brain areas **[17]**. Hence the level of serotonin and GABA neurotransmitters in subcortical regions of brain were determined in the present study.

The EPM test is predicted on the idea that exposure to an open arm of EPM elicits a conflict between approaching and avoiding that is noticeably stronger beyond the conflict elicited by exposure to an enclosed arm **[18]**. In our study, consistently significant increase in open arm entries and decrease in closed arm entries was observed on 7^{th}, 14^{th} and 21^{st} day in experimental animals treated with diazepam and both test extracts.

Animals finds the open arm unprotective and anxiogenic than closed arms. Hence animals prefer to enter and spend more time in closed arm than open arm. Anxiolytic drugs increase time spent in open arms **[19]**. The significant increase in time spent in open arms and decrease in time

spent in closed arms was observed on 7th, 14th and 21st day in experimental animals treated with diazepam and both test extracts.

The variables that are evaluated on EPM (Frequency of entries in open and closed arms, Time spent in open and closed arms) are integrated as anxiety index. The values of anxiety index ranges from 0-1. Higher anxiety-like behaviour is indicated by a rise in the anxiety index. Anxiolytic drugs show decrease in anxiety index **[20]**. An anxiety index is significantly decreased on 7th, 14th and 21st day in experimental animals treated with diazepam and both test extracts.

In LDT, mice typically avoid entering and engaging in exploratory behaviour in areas with bright lighting. Because they are afraid of being exposed to a new environment, animals always attempt to spend more time in a dark compartment than a light box. Anxiolytics usually lengthen the amount of time spent in a bright location **[21]**. In the present study the time spent in light area is significantly increased on 7th, 14th and 21st day in experimental animals treated with diazepam and both test extracts.

Because of habituation over time and the amount of time spent in each compartment, transitions have been said to be a measure of activity exploration. Drugs that reduce anxiety increase the frequency of transitions between the compartments.**[21]** In the present study significant ($p < 0.001$) increase in number of transitions observed in mice treated with diazepam and both test extracts when compared with the control group on 14th and 21st day of the experiment.

In the present study it was observed that mild stressor (inducer such as EPM and LDT) of anxiety reduced GABA level in the subcortical regions of the brain, in the control group. Experimental animals treated with both the extracts (100mg/kg and 200mg/kg) showed statistically significant ($p < 0.001$) increase in GABA level (28.28±0.61 and 33.43±0.99) when compared with control group (13.54±0.51). Animals treated by diazepam shows significant rise in GABA level (45.50±0.98).

Experimental animals treated with both the extracts (100mg/kg and 200mg/kg) showed statistically significant ($p < 0.001$) decrease in Serotonin level (52.73±1.43 and 43.02±0.98) when compared with control group (67.29±1.41). Animals treated by diazepam shows significant decrease in serotonin level (32.10±0.61). There is a decrease in level of the neurotransmitter 5-HT in both extract group which shows its anxiolytic effect.

CONCLUSION: -

Our research demonstrates that the *Carissa carandas* L. extract significantly affected the anxiety-related behavioural parameters

in mice exposed to the elevated plus maze test and the light-dark test. Extract from *Carissa carandas* L. produces "anxiolytic" behaviour. The increase in neurotransmitter such as GABA and decrease in level of serotonin in subcortical region of brain with *Carissa carandas* extract treatment was observed to be like that of the BDZ drug diazepam. All these effects could be attributed a number of phytoconstituents including flavonoids, triterpenoids and phytosterols which were found to be present in the extract. Overall, the findings show that the ethanolic extract of *Carissa carandas'* aerial portions has calming effects, probably as a result of its influence on serotonin and GABA receptors. Therefore, the extract might have a clinical use in the treatment of anxiety.

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