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Original Article

IN-VIVO PHARMACOLOGICAL INVESTIGATIONS OF BARK EXTRACTS OF *CARISSA CARANDAS*

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ABSTRACT

Objective: *Carissa carandas* is a species of flowering shrub in the dogbane family, Apocynaceae with a long history of traditional medicinal and agricultural uses; it is usually grown in all parts of Bangladesh. The methanol, ethanol and chloroform extracts of the plant bark was evaluated for preliminary phytochemical screening with its anti-pyretic activity, acute toxicity, GI motility, neurophramacological and anti-nociceptive activities.

Methods: The preliminary phytochemical analysis was performed on the basis of standard procedures. The anti-pyretic activity test was done by Brewer's yeast induced pyrexia model, acute toxicity test was performed by biometric evaluation, anti-nociceptive activity was tested by hot water tail immersion method and neuropharmacological activity tests were done by open field test and forced swimming test.

Results: Results showed that *Carissa carandas* bark extract has significant effects on the most of the activity tested except acute toxicity.

Conclusion: Therefore, the obtained results tend to suggest the neuropharmacological, anti-pyretic, anti-nociceptive and gastrointestinal motility activities of the three different solvent extracts of the plant barks and justify its use in folkloric remedies. This study also justifies further research to obtain more insight about this plant and compound isolation.

Keywords: *Carissa carandas*, Antipyretic activity, Anti-nociceptive activity, Neuropharmacological activities, Acute toxicity study.

INTRODUCTION

Owing to the global trend towards improved quality of life, there is great demand for medicinal plants in the developing world for treating various ailments of both man and animals. Recently, World Health Organization (WHO) estimated that 80% of people worldwide rely on herbal medicines partially for their primary health care. It has been recorded that about 450 to 500 plants growing or available in Bangladesh have therapeutic values [1, 2]. The plants are widely used by all sections of the society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional system- Ayurveda, Siddha, Unani [3].

Carissa carandas is a species of flowering shrub in the dogbane family, Apocynaceae however its botanical name was in recent years changed to *Carissa congesta* Wight. It grows everywhere in Bangladesh. Out climate is very suitable for the growth of this shrub. The unripe fruit used medicinally as an astringent. The ripe fruit is taken as an anti-scorbutic and remedy for biliousness [4]. The leaf decoction is valued in cases of intermittent fever, diarrhea, oral inflammation and earache. The root is employed as a bitter stomachic and vermifuge and it is an ingredient in a remedy for itches. The roots contain salicyclic acid and cardiac glycosides causing a slight decrease in blood pressure. Also reported are carissone; The D-glycoside of β-sitosterol; glucosides of odoroside H; carindone, a terpenoid; lupeol; ursolic acid and its methyl ester; also carinol, a phenolic lignin. Bark, leaves and fruit contain an unnamed alkaloid [5]. Phytochemical screening of the root extract showed that the crude extract contained small quantities of alkaloids, flavonoids, saponins and large amounts of cardiac glycosides, triterpenoids, phenolic compounds and tannins [6]. There are studies that corroborate the use of *Carissa carandas* in as anticonvulsant, astringent, hepatoprotective [6], analgesic antipyretic [3], and anti-diabetic, anti-microbial, anti-oxidant and anti-cancer agent [7]. So, it is highly relevant to investigate the in-vivo pharmacological activities of *Carissa carandas.* In the present study we aimed to explore and evaluate bioactivities including gastrointestinal motility test, anti-pyretic test, acute toxicity test, anti-nociceptive test and neuropharmacological activity test of the bark extract of *Carissa carandas.*

MATERIALS AND METHODS

Plant material collection and identification: Plant sample (bark) *Carissa carandas* was collected from Savar in May 2013 and submitted to the National Herbarium, Mirpur, Dhaka, Bangladesh for identification (Accession Number: DACB 37533).

Preparation of plant material*:* Bark were sundried for 7 days and later dried in drier at 40^0 C for about an hour. The dried bark were then ground into powder using high capacity grinding machine and stored in airtight plastic container with necessary markings for identification and kept in cool, dark and dry place for the investigation. According to Patil *et al.*, 2010 [8] the Bark of the plant material was extracted with methanol, ethanol and chloroform using soxhlet extraction apparatus for six to seven hours at a temperature not exceeding the boiling point of the solvent used. The extracts were named as methanol extract, ethanol extract and chloroform respectively.

Preparing animals*:* For the experiment Adult Swiss albino mice (BALB/c) weighing between (12-300) gm of either sex were collected from animal sources department of ICDDR, B, Dhaka. The animals were maintained under normal laboratory condition & kept in standard polypropylene cages at room temperature of 30°±2° C and 60% to 65% relative humidity and provided with standard diet & water. All protocols for animal experiment were approved by the institutional animal ethical committee.

Antipyretic activity test: Antipyretic activity test was done by Brewer's yeast induced pyrexia model. The mice were injected with brewer's yeast. Due to the presence of pyrogenic substances inside the body, pyrexia/fever occurred in the animals. All Albino Swiss mice were randomly divided into 8 groups containing 6 mice in each group and fasted for one hour before the experiment with free access to water. The body temperature of all the mices were measured by recording rectal temperature. Mice having normal temperature were selected for the test. Brewer's yeast suspension was injected subcutaneously in the mice. Rectal temperatures of the mices were recorded after 18 hours. Mice showing increase of temperature of at least 0.5°C to 1°C were selected for the experiment only [9]. Control solution (0.9% NaCl), standard drug (paracetamol 50 mg/kg) and sample solutions of 100 mg/kg and 200 mg/kg of all the extracts were administered by oral gavage. Rectal temperature were recorded after 60, 90, 120 min. Finally

the loss of temperature is measured in % reduction according to the following formula [10].

$$\% \text{ reduction} = \frac{\text{Yeast induced pyrexia} - \text{post treatment temperature}}{\text{Yeast induced pyrexia}} \times 100$$

Neuropharmacological study

Open field test: According to Gupta *et al.*, 1971 [11] with slight modification, we used open field test to monitor behavioral responses in mice that were placed in a novel and bright area. Rodents tent to stay away from brightly illuminated areas. The experiment also asses a range of anxiety- induces, locomotor activity and exploratory behaviors. The animals were pre treated according to the literature. The open field apparatus is made of hardboard (60 cm x 60 cm squares alternatively) and wall was 40 cm in height. Blue lines drawn on the floor and it was divided into 36 squares (10 cm x 10cm square alternatively) colored blank and white and centre square (10cm x 10 cm) in the middle clearly marked. The number of squares visited by the animals was calculated for 2 minutes at 0, 30, 60, 90, 120, and 150 subsequently to oral administration of the experimental crude extract.

Swimming test: According to Porsolt *et al.*, 1977 [12] with slight modification we used swimming test. Animal (e.g. Mice) were randomly divided into 8 groups where 6 mice on each group. Solutions were administered orally. The forced swim test was carried out on mice individually forced to swim in an open acquire water tank apparatus (29cm X 19cm X 20cm), containing 9cm of water at 25±1°C temperature. Each group of mice was administered methanol, ethanol and chloroform extract of *Carissa carandas* has given to mice as dose of 100 mg/kg and 200 mg/kg of body weight. The total duration of immobility during 6 min test was scored as described. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only that movement necessary to keep its head above. The duration of immobility was recorded. Decrease in the duration of immobility during the FST was taken as a measure of anti-depressant activity.

Acute toxicity test: The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment. The method as adopted in 1996 was extensively validated *in vivo* against LD50 data [13]. Mice were kept fasting for 1-2 hours but water was provided and were divided in 10 groups. Each group contained 6 mice. Then all the mice were marked and weighed. Control solution (0.9% NaCl), sample solution of each extract of 2000 mg/kg, 1000 mg/kg, 500 mg/kg were administered subcutaneously to the mice. Then the mice were kept on food deprivation for another 1-2 hours with access to water. All the mice were kept under observation for 14 days.

Gastrointestinal Motility test: The gastrointestinal motility test was performed according to Marona & Lucchesi, 2004 [14] with slight modifications where necessary. All the mice were kept in food deprivation for 3 hours but only accessible to water and they were divided in 8 groups, each group containing 6 mouse. Then the mice were weighed and marked. Control solution (5 ml/kg), standard solution (5 mg/kg), sample solutions of 100 mg/kg and 200 mg/kg of all the extracts were administered by oral gavage. The time of this dose administration is considered zero reading. 90 min later, 0.3 ml of charcoal suspension was administered to all the mice. After 60 minutes mice were provided with free access to food. Then the mice were observed at 5 min intervals until feces with charcoal were eliminated. Charcoal was observed on the feces using normal light when it was easily visible, or using a microscope to help the identification of the black spots. The results were based on the time for the charcoal to be eliminated.

Anti-nociceptive activity test (Tail immersion): The anti-nociceptive activity was performed by using hot water tail Immersion test [15]. According to this process, all the mice were kept in food deprivation for 24 hours but only accessible to water. Prior to analgesic experiments, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55° C – 55.5° C. The animal immersing the tail from hot water with in 5 second was selected for the study. The mice were divided in 8 groups, each group containing 6 mice. After marking and weighing all the mice the reaction time of all the mice was measured by immersing the distal part of tail (3.5 cm) in to hot water (55± 1° C). This reaction time is considered as zero reading. Control solution (5 ml/kg), standard solution (50 mg/kg), sample solutions of 100 mg/kg and 200 mg/kg of all the extracts of *Carissa carandas* were administered by oral gavage. The reaction time was then measured on 0, 30, 60, 90 and 120 minute after the administration of drug with the help of stop watch.

Statistical analysis

Data was expressed as Mean ± SEM (Standard error of Mean). The results were analyzed statistically by ANOVA followed by Dunnet's test. Results below $p<0.05$ and $p<0.01$ are considered statistically significant.

RESULTS AND DISCUSSION

Anti-pyretic Test: Methanol and ethanol extract of the bark of *Carissa carandas* contains alkaloids, carbohydrates, unsaturated sterols and saponins. They also contain flavonoid. Tannis is absent in the alcoholic extract of *Carissa carandas.* It is found that the higher dose (200 mg/kg body weight) of methanol and both doses of ethanol (100 and 200 mg/kg) showed accepting level of lowering pyrexia from elevated level which is shown in **figure 1**. It is observed from **figure 1** that chloroform extract shows significant lowering of basal temperature at 200 mg/kg body weight dose level. In **table 1** it is shown that higher dose of methanol, both doses of ethanol and chloroform at 100 and 200 mg/kg body weight extract dose showed maximum % reduction of temperature.

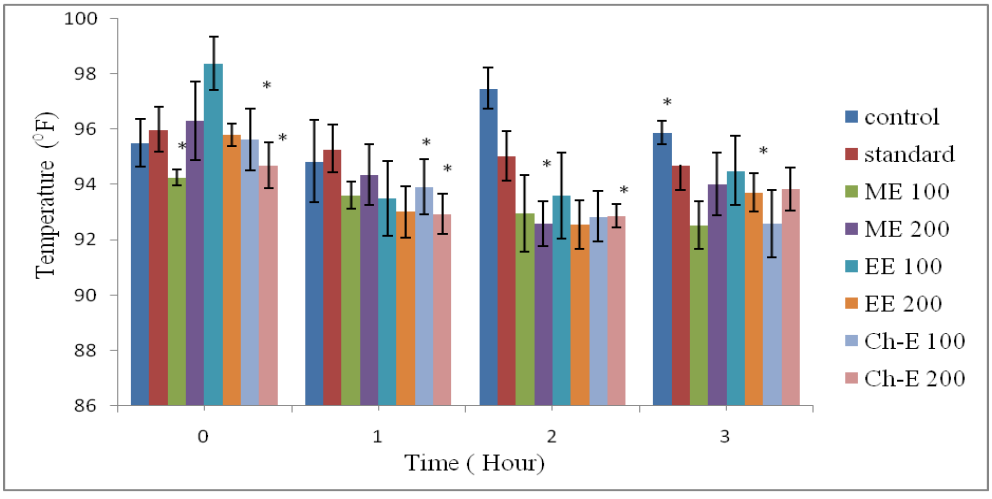

Fig. 1: Effect of bark extract of *Carissa carandas* in brewer's yeast induced pyrexia in mice.

Table 1: % Reduction of temperature using bark extract of *Carissa carandas*

Group	% reduction of temp (°F)		
	1hr % reduction	2hr % reduction	3hr % reduction
Control	0.0067±0.001	0.0091±0.002 **	0.0125±0.003
Paracetamol	0.0075±0.013	0.0202±0.007	0.0089±0.007
Methanol Extract 100	0.0052±0.004	0.0305±0.007 ***	0.0335±0.007 *
Methanol Extract 200	0.0198±0.008	0.0380±0.007	0.0237±0.015
Ethanol Extract 100	0.0497±0.006 *	0.0585±0.008	0.0394±0.006 *
Ethanol Extract 200	0.0290±0.008 *	0.0330±0.011	0.0216±0.007
Chloroform Extract 100	0.0179±0.003	0.0288±0.013 **	0.0283±0.014
Chloroform Extract 200	0.0185±0.004	0.0190±0.006	0.0117±0.003

Values are mean ± SEM (n=6), * (p< 0.05), ** (p< 0.01), *** (p< 0.001) significantly different when compared with the corresponding value of standard group, done by independent sample t-test.

In the present study, methanol, ethanol and chloroform extract showed antipyretic activities in mice. So we can say more of the active principles responsible for the antipyretic activity might be available in these three extracts. Brewer's Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermo-regulatory center at a lower temperature. So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of acetylsalicylic acid suggested that there are several mediators or multi-processes underlining the pathogenesis of fever .Inhibition of any of these mediators may bring about antipyretics. As to how they interfere with prostaglandin synthesis, further studies need to be carried out. Though this study could hint about the onset and duration of action of the extracts of the plants studied, further investigation is required to determine their pharmacokinetic profiles. The fact that neither toxicity nor lethality was observed at any dose of both extracts explains the wide safety margin of the extracts within the doses range. This observation also hints that the LD_{50} of the extracts is much higher than the highest dose level employed.

Table 2: Effect of different extract of *Carissa carandas* in open field test (Movement)

Group	Doses (mg/kg)	0 min	30 mins	60 mins	90 mins	120 mins	150 mins
Control		19.16±8.64	25.16±5.04	33±7.16	31.33±7.15	37.8±8.01	23.8±7.56
Std (Clonazepam)	2	13±1.82	24.5±5.05	27±5.97	22.83±6.03	24.16±3.28	15.16±2.79
Methanol Extract	100	41.83±10.55	45.33±18.76*	40.83±20.47	20.66±13.17*	40.66±35.20	21.5±5.18
	200	53.83±5.92	39±6.60	34.5±23.68	28.5±28.22	17±4.54	27.83±18.41
Ethanol Extract	100	61.16±18.48	53.5±28.51	42.66±24.36*	26.33±7.69	23.66±4.30*	17.33±8.03
	200	59.16±3.62	32.66±10.91	23.83±8.39	18.5±13.32	21.0±8.92	14±3.55
Chloroform Extract	100	38.16±14.25	33±6.16*	28.33±11.38	28.66±10.91*	22.16±8.53	23.33±8.29
	200	38.5±5.56	25.16±4.74	23.33±5.43	17.5±4.89	20.5±9.08	22.2±6.05

Values are mean ± SEM (n=6), * (p< 0.05), significantly different when compared with the corresponding value of standard group, done by independent sample t-test.

Table 3: Effect of different extract of *Carissa carandas* in open field Test (Standing)

Group	Doses (mg/kg)	0 min	30 mins	60 mins	90 mins	120 mins	150 mins
Control		0.16±0.37*	2.83±2.19	3.5±1.97*	3.83±2.19	2.83±2.26	2.16±1.57
Std (Clonazepam)	2	1.33±1.37	6.83±1.46	5±1	5.16±1.34	4.16±0.68	4.33±1.97
Methanol	100	3.0±6.71	6±2.99	4.5±5.80	3.5±2.68	4.5±2.06	7.5±2.30
Extract	200	11.25±4.91	5.5±1.99	4.5±5.32	3.75±1.67	3.25±2.26	4.0±2.62
Ethanol	100	8.83±8.35	10.33±4.78	9.0±4.54	5.16±2.26	4.83±1.34	3.83±1.95
Extract	200	7.83±4.81	10.66±4.34	6.0±2.38	4.33±1.88	5.50±1.89	5.33±3.39
Chloroform Extract	100	12.33±4.42	10.33±6.10	13.66±3.39	14.16±4.81	12.66±1.10	14±1.73
	200	13.66±4.18	14±1.63	13.83±1.95	13.5±1.25	13±2.0	14.16±1.67

Values are mean ± SEM (n=6), * (p< 0.05), significantly different when compared with the corresponding value of standard group, done by independent sample t-test.

Table 4: Effect of different extract of *Carissa carandas* in open field test (Centre)

Group	Doses (mg/kg)	0 min	30 mins	60 mins	90 mins	120 mins	150 mins
Control		1±0.57	0.66±0.74	0.5±1.60	0.33±0.98	0.5±1.6	0.5±1.04
Std (Clonazepam)	2	0.66±0.47	0.66±1.0	0.5±0.5	1.0±0.57	0.33±0.47	0.66±0.47
Methanol	100	2.16±1.57	1±0	0.83±0.68	1.16±1.06	0.83±0.68	0.83±0.68
Extract	200	1.66±1.10	2.16±1.77	1.0±1.15	1.16±1.06	0.83±0.68	0.83±0.68
Ethanol	100	2.33±1.37	0±2.05*	1.16±0.68*	0±0	1.5±0.95*	1.0±0.81
Extract	200	2.5±1.70	2.16±1.34	0.5±0.76	1.16±0.89	1±1	1±0.81
Chloroform Extract	100	1.16±0.68	0±0	1.83±3.23	0.66±0.74	0.33±0.47	0.5±0.5
	200	1.33±0.47	0±0	0.5±0.76	0±0	0.83±0.68	0±0

Values are mean ± SEM (n=6), * (p< 0.05), significantly different when compared with the corresponding value of standard group, done by independent sample t-test.

Table 5: Effect of different extract of *Carissa carandas* in open field test (Stool):

Group	Doses (mg/kg)	0 min	30 mins	60 mins	90 mins	120 mins	150 mins
Control		0.83±0.68	0±0	0±0	0±0	0±0	1.67±1.21
Std (Clonazepam)	2	1.0±0.81	0±0	0±0	0±0	0.66±0.74	0.33±0.47
Methanol	100	1±0.81	0.83±1.06	0.66±0.74	0±0	0.83±0.68	0.66±0.74
Extract	200	0.83±0.68	1.5±1.38	0±0	0±0	1±0.81	0.66±0.74
Ethanol	100	0.5±0.76	0.5±0.5	0±0	0±0	0.66±0.74	0.83±0.68
Extract	200	1.33±0.47	0±0	0±0	0±0	0.33±0.47	0.5±0.76
Chloroform	100	0.83±1.06	0.5±0.5	0.5±0.5	0±0	0.66±0.74	1.0±0.81
Extract	200	1.16±0.68*	0.66±0.74*	0.66±0.74	0.83±0.89	1.0±1.41	0.83±0.68*

Values are mean ± SEM (n=6), * (p< 0.05), significantly different when compared with the corresponding value of standard group, done by independent sample t-test.

Neuropharmacological Study

Open field test*:* Test results for different extracts of *Carissa carandas* for open field test (movement), open field test (standing), open field test (center) and open field test (Stool) are presented in **table 2, 3, 4 and 5** respectively.

From those tables we can see that, Methanol extract of *Carissa carandas* showed enough effect like standard (diazepam) in dose dependent like manner. It increases the movement, entrance into centre and standing. Chloroform also showed diazepam like effect but could not reach significances whereas diazepam decreased movement significantly (p<0.05).Among all parameters ethanol did not show diazepam like effect on defecation which is shown in **table 5.**

According to Bronikowski *et al.*, 2001 [16] measuring aspects of rat behavior in a contained arena would indicate the emotional reactivity of the subjects. Many reports have validated open field tests as useful measures of emotional activity [17, 18] reviewed by Sandnabba, 1996 [19] for Turku aggressive mice; others have not found differences in open field activity despite difference in other anxiety measures e.g, MHC-congenic mice. The standard open field test is commonly used to assess locomotor, exploratory and anxiety like behavior in laboratory animals (rats/mice) [20]. The open field test is designed to examine responses of mice or rats to a new and unfamiliar environment (novel environment). Rodents demonstrate anxiety, fear and curiosity when placed in a new environment [21]. In response to the novel environment the rodents tend to explore the surrounding. The exploratory capacity might be considered to be an index of anxiety although it is difficult to separate it from motor anxiety [21]. However, rodents are also fear to go to the open and illuminated space which is clearly demonstrated by their rearing, grooming, defecation, locomotor and so on. These parameters are well utilized to assess anxiety and fear in rodents.

Inhibition of such behaviors is indicative of centrally acting depressant or sedatives [21]. Here **tables 2 to 5** represent the effect of different extract of *Carissa carandas* on various parameters of open field test. Chloroform extract decreased movement of rodents in a dose dependent manner but could not reach significant. Whereas, clorazepam decreased movement significantly. Ethanol increased the movement and chloroform increased the standing. Ethanol extract increased the open field. The effect of lower dose of methanol (100 mg/kg b.w.), ethanol (100 mg/kg b.w.) and chloroform (100 mg/kg) on defecation was like standard (clonazepam). The results show that the methanol and ethanol extract has not the ability to relieve stress and had an anxiolytic effect on the rodent.

Swimming test: The result of different bark extracts of *Carissa carandas* in mice in forced swimming test is represented by **table 6**. During the test the methanol extract at doses of 100 mg/kg & chloroform extract at doses of 100 & 200 mg/kg of body weight shortened the immobility period in comparison with control & exhibited a dose dependent antidepressant activity. A significant (p<0.05) decrease in duration of immobility was observed as compared to that of control. The result shows that ethanol extract of *Carissa carandas* does not pose any antidepressant activity.

Table 6: Effect of different extract of *Carissa carandas* in swimming test

Group	Doses (mg/kg)	Duration of Immobility (s)
Control		34.22 ± 2.42
Std (Imipramine)	10	37.75 ± 0.34
Methanol Extract	100	39.22 ± 0.22 *
	200	37.61 ± 0.62
Ethanol Extract	100	39.5 ± 0.67
	200	40.5 ± 1.27
Chloroform Extract	100	37.58± 0.49 *
	200	37.05 ± 0.60 *

Values are mean ± SEM (n=6), * (p< 0.05), significantly different when compared with the corresponding value of control group, done by independent sample t-test.

Forced Swimming Test (FST) was designed by Porsolt *et al.*, 1978 [22] as a primary screening test or anti-depressant. It is still one of the best models for this procedure. This is a low-cost, fast and reliable model to test potential anti-depressant treatment with a strong predictive validity. However, the low face and construct validities should not forbid the use of this model for neurophysiological studies. It has a great sensitivity with all the antidepressant classes and all the mechanisms of action of treatments could be determined, but clinical correlations should be considered very carefully. When rodents are forced to swim in a confined place, they tend to become immobile after vigorous activity. This stressful inescapable situation can be evaluated by assessing different behavioral strategies and immobility during the test could be an efficient adaptive response to the stress [22]. The development of immobility when the rodents are placed in an inescapable container of water reflects the cessation of persistent escape directed behavior [23]. The CNS depressant effect of the extract may be attributed to chemical constitute other than flavonoids and alkaloids because flavonoids are responsible for the decrease in immobile phase in the swim test [24] and so does alkaloid as well [25].

Acute toxicity test: In the time of investigation of acute toxicity none of the extracts showed any sign of toxicity in the period of one week observation which is shown in **table 7**. Hegde & Joshi, 2009 [6] described that oral administration of ethanol extract of leaves of *Carissa carandas* produce no visible signs of toxicity.

Table 7: Acute Toxicity test of different extracts of *Carissa carandas* on mice

Solution	Group	Dose (b.w)	No. of deaths
Control (0.9% NaCl)	G-1	0.5ml/100gm	none
Methanol Extract	G-2	2000 mg/kg	none
	G-3	1000 mg/kg	none
	G-4	500 mg/kg	none
	G-5	2000 mg/kg	none
Ethanol Extract	G-6	1000 mg/kg	none
	G-7	500 mg/kg	none
Chloroform Extract	G-8	2000 mg/kg	none
	G-9	1000 mg/kg	none
	G-10	500 mg/kg	none

Table 8: Gastrointestinal motility determination of different extracts of *C. carandas*

Groups	Treatment	Dose	Time of charcoal defecation(min)
G-1	**Control (0.9% NaCl)**	5 ml/Kg	89.2±5.48
G-2	**Standard**	Butapen (5 mg/kg)	138.2±4.55
G-3	**Methanol Extract**	100 mg/kg	120.8±6.38**
G-4		200 mg/kg	115.6±5.58**
G-5	**Ethanol Extract**	100mg/kg	119.6±5.94**
G-6		200mg/kg	122±7.80*
G-7	**Chloroform Extract**	100 mg/kg	125.4±3.12***
G-8		200mg/kg	119.4±3.512**

Values are mean ± SEM (n=6), * (p< 0.05) ** (p< 0.01) and *** (p< 0.001) significantly different when compared with the corresponding value of control group, done by independent sample t-test.

Table 9: Anti nociceptive Activity Test (Tail immersion) of different extracts of *Carissa carandas*

Group	Treatment	Dose	0 min	30 min	60 min	90 min	120 min
G-1	**0.9% NaCl**	0.5ml/100 gm	9.66±1.53	12.91±2.13	10.89±1.14	10.30±0.67	9.59±0.58
G-2	**Diclofenac Na**	50 mg/kg	6.82±1.56	12.09±3.42**	14.64±5.82	12.30±4.10	9.82±4.00
G-3	**Methanol Extract**	100 mg/kg	12.22±1.99	9.43±1.51	5.30±1.13**	5.75±2.08	4.15±0.98***
G-4		200 mg/kg	15.07±4.21*	5.46±1.55**	8.92±5.31	7.5±3.51	7.14±3.55*
G-5	**Ethanol Extract**	100 mg/kg	8.36±1.15	5.21±1.48*	5.14±1.02**	3.57±0.90**	3.99±0.85***
G-6		200 mg/kg	8.14±1.49	14.19±2.58	14.29±2.01	20.43±2.45**	12.45±0.87*
G-7	**Chloroform Extract**	100 mg/kg	3.24±0.56**	11.81±1.94	8.03±1.17	15.22±1.34**	9.28±0.63
G-8		200 mg/kg	4.94±0.75*	10.52±1.24	13.55±0.69	13.40±1.15*	14.63±0.67***

Values are mean ± SEM (n=6), * (p< 0.05), ** (p< 0.01) and *** (p< 0.001) significantly different when compared with the corresponding value of control group, done by independent sample t-test.

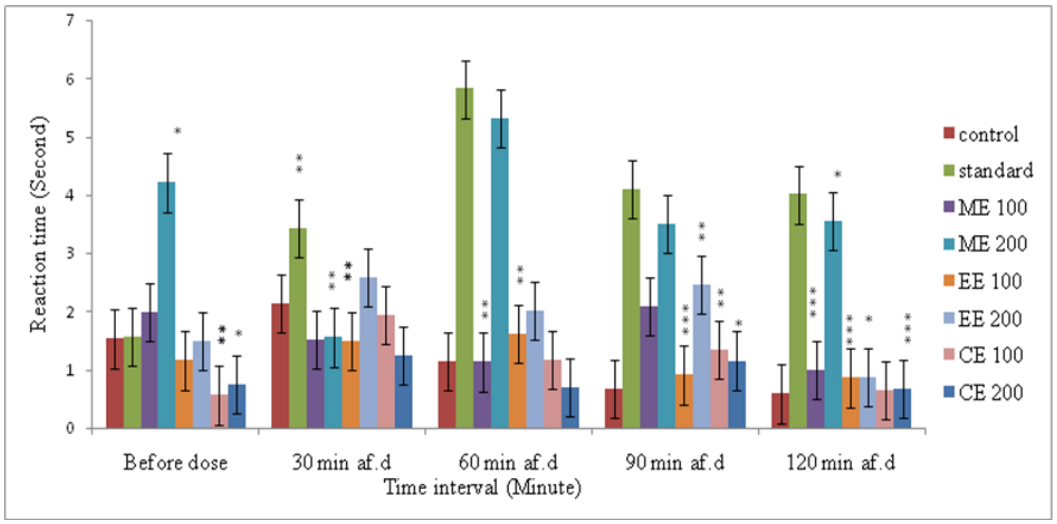

Fig. 2: Comparative graphical representation of anti nociceptive activity test of *Carissa carandas* in tail immersion test.

Gastrointestinal motility test: The gastro-intestinal motility test results are shown in **table 8**. In this test, methanol, ethanol and chloroform extract at doses of 100 and 200 mg/kg body weight were administered and all the extracts in both doses showed significant result compared to control in GI motility test. The results revealed that this drug caused a significant decrease in gut motility, compared

with the effect produced by normal saline. The time of charcoal elimination in treated mice and time of charcoal elimination in control mice were significantly different. The marked decrease in the propulsive movements generated by butapen could be determined by the observed presence of charcoal in the animal feces.

Anti-nociceptive activity (Tail immersion test): The results of anti-nociceptive activity by tail immersion test are presented by **table 9.** In this test, methanol, ethanol and chloroform extract at doses of 100 and 200 mg/kg body weight were administered and all the extracts in both doses showed significant analgesic effects after different time intervals. A comparative graphical representation of the test results are shown in **figure 2.**

In tail immersion method, the heat itself acts as a source of pain. The difference concentrations of extract of plant (100 and 200 mg/kg) and diclofenac sodium (50 mg/kg) was administered to mice and observed the basal reaction time in different time intervals. The basal reaction time increases with increasing the concentrations along with increasing the time. The basal reaction time is more for standard drug when compared to plant extracts. In case of plant extracts the ethanol (200 mg/kg) and chloroform (100 and 200 mg/kg) showed less reaction time and is covered after 120 min.

The higher dose of methanol showed more reaction time as compared to standard. The order of potency in 30 min after dose administration is ethanol › standard › control › chloroform › methanol. Lower doses of methanol and ethanol showed significant result as compared to control ($p<0.05$).Chloroform showed more significant ($p<0.001$) result in dose dependent manner significantly different from control.

CONCLUSION

From this study, it can be concluded that *Carissa carandas* bark extract of three solvents (methanol, ethanol and chloroform) show antipyretic effect. It is shown that higher dose of methanol, both doses of ethanol and chloroform at 100 and 200 mg/kg body weight extract dose showed maximum % reduction of temperature. None of extract has any kind of toxicity. In the result of gastrointestinal motility test the charcoal defecation time of methanol 100 mg/kg and ethanol 100 mg/kg body weight is similar chloroform extract has significant effect ($p<0.001$) on gastrointestinal motility . In open field test methanol extract of *Carissa carandas* showed enough effect like standard (diazepam) in dose dependent like manner. Methanol and chloroform show anti-depressant effect. In swimming test methanol and chloroform extracts showed decrease immobility with dose dependently like the standard imipramine which indicates their anti-depressant effect. Our current work is suggestive to future works on *Carissa carandas* with a consideration of compound isolation for particular activity and develop lead compound for therapeutic use.

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