IJBPAS, January, 2021, 10(1): 254-262

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

BIOCHEMICAL AND HEMATOLOGICAL STUDIES OF *CARISSA CARANDAS* IN EXPERIMENTAL ANIMAL MODEL

KUMARI P[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: Parveen Kumari: E Mail: parveen.punia7798@gmail.com**

Received 2nd April 2020; Revised 21st April 2020; Accepted 14thJuly 2020; Available online 1st Jan. 2021

https://doi.org/10.31032/IJBPAS/2021/10.1.5335

ABSTRACT

Carissa carandas (Apocynaceae) is a species of flowering shrub. The plant bears a berry- sized fruits which is commonly used as a condiment and additive to pickles and spices. The fruits, leaves, barks, and roots of *Carissa carandas* have been used for ethnopharmacologically. Chemical constituents mainly reported are steroids, terpenes, tannins, flavonoid, benzenoids, phenylpropanoid, lignans, sesquiterpenes, and coumarins.

Carissa carandas has been shown to have anticonvulsant effects via non-specific mechanisms since it reduced the duration of seizures produced by maximal electroshock as well as delayed the latency of seizures produced by pentylenetetrazole and picrotoxin induced seizures. In the present study ethanolic extracts of fruit of *Carissa carandas* was screened for various hematological and biochemical effects with the hope of finding safe and effective natural medicines.

The effect of *Carissa carandas* extract on biochemical parameters (Blood glucose, AST, ALT, Urea, Creatinin and bilurubin) showed that the plant did not affect most of these parameters except the ALT and Urea levels. The different doses of ethanolic extract of *Carissa carandas* produced a significant reduction in ALT level in a dose dependent manner.

Administration of etahnolic fruit extract extracts of *Carissa carandas*, showed no sign of toxicity in experimental animals and can be used safely.

Keywords: *Carissa carandas,* Biochemical, Hematological, Toxicity

INTRODUCTION

Central nervous system (CNS)-related disorders are becoming common day by day with modernization of society **[1]**, as a result of which individuals depend intensely on prescriptions drugs that help in treat these kind of disorders and one of them is anxiety, benzodiazepines class of drugs which are highly recommended for anxiety disorders, however their utilization is constrained due to clinically demonstrated unfavorable impacts like, psychomotor weakness, potentiating other central depressant medications, and reliance **[2-3]**. Along these lines, the quest for new and safe medications having anxiolytic properties that are free of the intricacies of benzodiazepines would be vital in the treatment of anxiety-related disorders. It is a well known and established fact plant extracts and natural products isolated from them are safe **[4]**. *Carissa carandas* (Apocynaceae) is a species of flowering shrub. The plant bears a berry-sized fruits which is commonly used as a condiment and additive to pickles and spices. It is a hardy, drought-resistant plant thriving well in a wide range of soils. Common names include Koromcha in Bangla and Karanda in English **[5]**. The fruits, leaves, barks, and roots of *Carissa carandas* have been used for ethnopharmacologically in the treatment of diarrhea, stomachic, anorexia, intermittent fever, mouth ulcer and sore throat, syphilitic pain, burning sensation, scabies, and epilepsy **[6]**. Chemical constituents mainly reported are steroids, terpenes, tannins, flavonoid, benzenoids, phenylpropanoid, lignans, sesquiterpenes, and coumarins **[7]**. *Carissa carandas* has been shown to have anticonvulsant effects via non-specific mechanisms since it reduced the duration of seizures produced by maximal electroshock as well as delayed the latency of seizures produced by pentylenetetrazole and picrotoxin induced seizures which can be attributed to its cns activity, but there are no research findings about its neuropharmacological effects **[8]**. Moreover, to find a drug free from toxic effects is hard to find making herbal medicine a preferred source of medicine. Therefore, methanolic extracts of fruit of *Carissa carandas* was screened for various hematological and biochemical effects with the hope of finding safe and effective natural medicines.

PLANT MATERIAL

The Fresh fruits of *Carissa carandas* Linn. Plant were collected, identified by local farmers and authenticated by botanist.

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 **[9-10]**.

ANIMALS

Male albino rats of wistar strain weighing between 100 – 160 g were used and maintained under laboratory conditions: humidity, temperature (23 - 25°C) and light 12 h light/dark cycle and were allowed free accesses to feed and water ad libitum. The animals were acclimatized for four weeks. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee.

ACUTE TOXICITY STUDY [11]

Acute toxicity study of ethanolic fruit extract of *Carissa carandas* (EECC) was determined in Wistar albino rats (150-180 g) according to OECD guidelines No. 425. The animals were fasted overnight and EECC was administered orally with a starting dose of 2000 mg/kg, to different groups of animals. Animals were observed continuously for first 3 h and monitored for 14 days for mortality and general behaviour of animals, signs of discomfort and nervous manifestations

SUBACUTE TOXICITY TEST [12-13]

Twenty-eight male albino Wistar rats were used for the study. The rats were divided into four groups of seven rats per group.

Group I- Control received 0.9% NaCl

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

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

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

All the animals received dosing schedule for 16 days via gastric intubation. Furthermore, animals in group 1 (control group) did not receive the extract, but were treated with normal saline. Animals were weighed on initial (0) day, 3rd, 6th, 9 th and 14th day

during the period of administration. After this period, the rats were subjected to overnight fast. The rats were subsequently anaesthetized with diethyl-ether and blood sample will be collected by cardiac puncture into EDTA and lithium heparin bottles. The heparinized samples were centrifuged at 3,000 x g for 10 min to obtain the plasma and stored at - 20°C until ready for analysis; while the whole blood samples were maintained at 4°C, the plasma glucose level was determined immediately.

Serum obtained was used to study hematological parameters (Total WBC, hemoglobin, PCV and Platelet count) and biochemical parameters (Blood glucose, AST, ALT, Urea, Creatinin and bilurubin) through established protocols **[14-19]**.

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. www.graphpad.com

RESULT

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.

The animals stayed healthy throughout all the trials. Administration of etahnolic fruit extract extracts of *Carissa carandas*, showed no sign of toxicity in experimental animals.

A weekly body weight was determined on initial (0) day, 3rd, 6th, 9 th and 14th day of four groups. The first one is the control, Group I is EECC of 250 mg/kg, II EECC of 500 mg/kg, and the last group, named as Group III, is EECC of 1000 mg/kg. No significant changes in the body weight were observed **(Table 1)**. The effects of subacute administration of EECC on haematological parameters are presented in **Table 2**. Treatment with EECC doesn't significantly modified parameters, like haemoglobin and PCV. But significantly ($p<0.01$) altered the parameters like total white blood cell count and platelet count in treated rats as compared to control group at the dose of 500 mg/kg and 1000 mg/kg. The effects of subacute administration of EECC on biochemical parameters are presented in **Table 3**. The EECC had no effect on the kidney function parameter, like creatinine, and but significant ($p<0.01$) changes were observed in level of urea. No statistically significant differences in the liver function parameter like aspartate aminotransferase (AST) were observed. On

the other hand there was significant decrease ($p<0.01$) in level of alanine aminotranferase (ALT) in EECC treated animals ate the dose of 500 mg/kg and 1000 mg/kg. Additionally, no relevant changes were found in albumin and bilirubin levels.

Table 1: Effect of EECC on haematological parameters in the subacute oral toxicity study

Parameter/Groups	Unit	Control	I-EECC (250 mg/kg)	II-EECC (500 mg/kg)	III-EECC (1000 mg/kg)
Haemoglobin	gm/dl	10.14±0.5	10.85±0.4	11.42±0.36	10.72±0.42
Packed Cells Volumes	10^3/µl	632.43±0.8	621.14±0.9	564.86±2.3	558.86±2.2
Total White Blood Cells	10^3/µl	4.59±0.05	4.64±0.06	4.56±0.06**	4.52±0.07**
Platelet count	10^3/µl	324.29±0.5	320.29±0.4	364.4±2.6**	384.29±0.4**

Values are given as mean±S.E.M. from seven rats each group, one-way ANOVA followed by Dunnett's 't' test. * p < 0.05 significant from control animals, ** p < 0.01 significant from control animals

Table 2: Effect of EECC biochemical parameters in the subacute oral toxicity study

Parameter	Unit	Control	I-EECC(250 mg/kg)	II-EECC (500 mg/kg)	III-EECC (1000 mg/kg)
Ast	U/l	23.71±0.68	22.85±0.5	22.42±0.36	22.28±0.74
Alt	U/l	36.71±0.6	34.57±0.8	32.28±0.6**	22.28±0.91**
Albumin	gm/dl	3.75±0.06	3.82±0.21	3.62±0.12	3.54±0.07
Creatinin	mmol/l	0.93±0.02	0.71±0.04	0.70±0.09	1.11±0.15
Urea	mmol/l	7.95±0.22	7.56±0.27	5.58±0.35**	5.32±0.47**
Bilurubin	mg/dl	0.22±0.01	0.20±0.12	0.21±0.05	0.19±0.03

Values are given as mean ± S.E.M. from seven rats each group, one-way ANOVA followed by Dunnett's 't' test. * p < 0.05 significant from control animals, ** p < 0.01 significant from control animals

Table 3: The effect of EECC on body weight of rats (g) at different days

Group	Treatment	Body weight (gm)				
		Initial day	Day 3	Day 6	Day 9	Day 14
Control	0.9 Nacl	132.20±0.02	137.44±0.25	142.62±0.76	145.54±0.85	147.22±0.16
I	EECC(250 mg/kg)	136.21±0.22	142.36±0.79	145.17±0.20	152.34±0.74	153.22±0.23
II	EECC (500 mg/kg)	140.20±0.29	146.19±0.04	148.22±0.98	152.67±0.81	152.18±0.24
III	EECC (1000 mg/kg)	140.26±0.24	142.12±0.85	150.34±0.01	151.23±0.02	152.78±0.81

Group I-III: ethanolic extract with different doses; Values are given as mean±S.E.M. from seven rats each group, one-way ANOVA followed by Dunnett's 't' test. * p < 0.05 significant from control animals, ** p < 0.01 significant from control animals

DISCUSSION

Synthetic psychoactive drugs are widely used in the management of central nervous system (CNS) related disorders **[20]**. However, their nonstop and aimless use has prompted adverse effects influencing the endocrine, autonomic, hypersensitive, hematopoietic, and neurological frameworks of the human body **[21]**. Therefore, researchers are scanning for new remedial drugs with least reactions and maximum potency from therapeutic plants, which are accepted to be protected and financially viable, also there is an exponential increase in screening botanicals for (CNS) related disorders **[22]**.

The animals stayed healthy throughout all the trials this might be due to the food value of the extracts, which can be a good source of various nourishment. EECC at all dosages were safe and animals showed symptoms of

alertness followed by relaxation or reduced activity. Administration of EECC, showed no sign of toxicity in experimental animals.

Evaluation of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. Such toxicity testing is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity, when data are translated from animal studies **[23]**. It can also be used to explain blood relating functions of chemical compounds/plant extract **[24]**. In sub acute toxicity studies hematological parameters such as Total WBC, hemoglobin, PCV and Platelet count were studied concentration, Administartion of EECC did not show any significant changes (Table 1). Except the levels of Platelet count which were increased significantly ($p<0.01$). this may be due to the anti-inflammatory activity of plant**[25]** these studies are in accordance with the research done on case of papaya leaf extract**[26]**.

The effect of *Carissa carandas* extract on biochemical parameters (Blood glucose, AST, ALT, Urea, Creatinin and bilurubin) showed that the plant did not affect most of these parameters except the ALT and Urea levels. High level of transaminases such as ALT and AST is a sign for hepatic damage **[27-28]** and are measured clinically as a part of a diagnostic evaluation of liver function test. The different doses of ethanolic extract of *Carissa carandas* produced a significant reduction in ALT level in a dose dependent manner. The change in the level of urea, which was increasing to significant value. These elevated values may be due to effect of *Carissa carandas* on amino acid synthesis and degradation, which lead to increase the uses of glucose as source of energy instead of protein and result in preserve the protein **[29]**.

CONCLUSION

CNS acting synthetic drugs are the main source of drug prescribed for the management of central nervous system (CNS) related disorders. But, they have their own adverse effects. As a result of this there is a increase a tremendous increase in use of herbal drugs for the management of various disorders. Therefore, researchers are trying to find out drugs with least reactions and maximum potency from therapeutic plants, which are accepted to be safe and financially viable.

Investigations on the phytochemical screening of *Carissa 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 *Carissa carandas* could be mediated by synergistic action of these phytochemicals.

The animals stayed healthy throughout all the trials. Administration of etahnolic fruit extract extracts of *Carissa carandas*, showed no sign of toxicity in experimental animals.

REFERENCES

[1] Hidaka BH, Depression as a disease of modernity: Explanations for increasing prevalence. Journal of Affective Disorders, 140(3), 2012, 205–214.

[2] Uzun S, Kozumplik O, Jakovljević M, Sedić B, Side Effects of Treatment With Benzodiazepines. Psychiatria Danubina, 22(1), 2010, 90-93.

[3] Gudex C, Adverse effects of benzodiazepines. Soc Sci Med, 33(5), 1991,587.

[4] Thakur P and A.C. Rana AC, Anxiolytic potemntail of medicinal plants. Int J Nutr Pharmacol Neurol Dis, 3, 2013, 325-31.

[5] Morton J (1987) Fruits of Warm Climates. Miami, FL: Julia F. Morton, pp: 5-11.

[6] Begum S, Saqib AS, Bina SS, Carandinol: first isohopane triterpene from the leaves of Carissa carandas L. and its cytotoxicity against cancer cell lines. Phytochemistry Letters, 6, 2013, 91–95.

[7] Ya'u J, Yaro AH, Abubakar MS, Anuka JA, Hussaini IM, Anticonvulsant activity of Carissa edulis (Vahl) (Apocynaceae) root bark extract. Journal of Ethnopharmacology, 120(2), 2008, 255–258.

[8] Hegde K, Thakker S, Joshi A, Shastry C, Chandrashekhar K, Anticonvulsant Activity of Carissa carandas Linn. Root Extract in Experimental Mice. Tropical Journal of Pharmaceutical Research, 8(2), 2009.

[9] Sofowora A,Medicinal plants and traditional medicine in Africa. Spectrum books Ltd, Ibadan Nigeria. 1993, 150.

[10] Evans WC, Trease and evans' pharmacognosy. E-book. Elsevier Health Sciences. 2009, 176- 180.

[11] Organization for Economic Cooperation and Development, 2004. Guidelines for testing of chemicals (OECD-423).

[12] Das N, Goshwami D, Hasan MS, Raihan SZ, Evaluation of acute and subacute toxicity induced by methanol extract of Terminalia

citrina leaves in Sprague Dawley rats. Journal of Acute Disease, 4 (4), 2015, 316–321.

[13] Adebayo AH, Abolaji AO, Opata TK, Adegbenro IK, Effects of ethanolic leaf extract of Chrysophyllum albidum G. on biochemical and haematological parameters of albino Wistar rats. African Journal of Biotechnology, 9(14), 2010, 2145-2150.

[14] Reitman S, and Frankel S, Method for serum, using the colorimetric SGOT/SGPT assay. Am J Clin Pathol, 28, 1957, 56.

[15] Jaffe M, Hoppe-Seylers Z. physiol, Chem, 10, 1886, 391.

[16] Doumas BT, Watson WA, Biggs HG, Albumin standards and the measurement of serum albumin with bromcresol green. Clinica chimica acta, 31 (1), 1971, 87-96.

[17] Doumas BT, Perry BW, Sasse EA, Straumfjord JV, Standardization in bilirubin assays: evaluation of selected methods and stability of bilirubin solutions. Clinical chemistry, 19(9), 1973, 984-993.

[18] Searcy RL, Reardon JE, Foreman JA, Enzymatic serum urea determination. Am. J. Med. Technol, 33, 1967, 15-20.

[19] Cheesbrough M, District laboratory practice in tropical countries. Cambridge university press, 2006, 311-340.

[20] Croxford JL, Therapeutic Potential of Cannabinoids in CNS Disease. CNS Drugs, 17(3), 2003, 179–202.

[21] Kaminer Y, Goldberg P, Connor DF, Psychotropic Medications and Substances of Abuse Interactions in Youth. Substance Abuse, 31(1), 2010, 53–57.

[22] Graziano S, Orsolini L, Rotolo MC, Tittarelli R, Schifano F, Pichini S, Herbal Highs: Review on Psychoactive Effects and Neuro-pharmacology. Current Neuro-pharmacology, 15(5) 2017.

[23] Clark M and Steger-Hartmann T, A big data approach to the concordance of the toxicity of pharmaceuticals in animals and humans. Regulatory Toxicology and Pharmacology, 96, 208, 94–105.

[24] Yakubu MT, Akanji MA, Oladiji AT, Hematological evaluation in male albino rats following chronic administration of aqueous extract of

Fadogia agrestis stem. Pharmacogn. Mag, 3, 2007, 34-38.

[25] Anupama N, Madhumitha G, Rajesh KS, Role of Dried Fruits of Carissa carandas as Anti-Inflammatory Agents and the Analysis of Phytochemical Constituents by GC-MS. BioMed Research International, 2014, Article ID 512369.

[26] Dharmarathna SL, Wickramasinghe S, Waduge RN, Rajapakse RP, Kularatne SA, Does Carica papaya leaf-extract increase the platelet count? An experimental study in a murine model. Asian Pacific journal of tropical biomedicine, 3(9), 2013, 720–724.

[27] Lott JA and Wolf PA, Alanine and Aspartate Aminotransferase (ALT and AST), In: Clinical Enzymology: A Case-Oriented Approach, Lott, J.A. and P.L. Wolf (Eds.). Field, Rich and Associates, New York, USA. 1986, 111-138.

[28] Cordoba J, O'Riordan K, Dupuis J, Borensztajn J, Blei AT, Diurnal variation of serum alanine transaminase activity in chronic liver disease. Hepatology, 28, 1998, 1724-1725.

[29] Alkhateeb H and Bonen A, Thujone, a component of medicinal herbs, rescues palmitate-induced insulin resistance in skeletal muscle. Am. J. Physiol.-Regul. Integr. Comp. Physiol, 299, 2010, R804-R812.