The Journal of Phytopharmacology 2020; 9(4): 265-269
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2020; 9(4): 265-269
July- August
Received: 21-05-2020
Accepted: 11-07-2020

doi: 10.31254/phyto.2020.9409

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Acute and subacute toxicity evaluation of aqueous extracts of *Carpobrotus edulis* in Sprague Dawley rats

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ABSTRACT

Carpobrotus edulis is a common medicinal plant used in Southern Africa. Despite its extensive use in herbal medicine, there is no documented scientific evidence corroborating its safety. This study aims to evaluate the acute and subacute toxic effects of the aqueous extracts of *Carpobrotus edulis* in Sprague Dawley rats. In acute toxicity testing, three healthy female Sprague Dawley rats were exposed to *Carpobrotus edulis* aqueous extract per step at any of the four fixed dose levels of 300, 600, 1200 and 2000mg/Kg body weight. The Sprague Dawley rats were observed clinically for any signs of toxicity. A 28-day subacute toxicity testing was carried out on thirty-two Sprague Dawley rats grouped in four experimental groups of eight animals each. Group A received 100mg//kg of the extract, Group B received 300mg/kg while Group C received 1000mg/kg. Group D was a negative control group and received distilled water. Bodyweight, feed and water intake were measured at weekly intervals. Blood for biochemical analysis was collected on the last day of the study period. Gross pathological and histopathological examination was done on all experimental rats. There were no clinical signs suggestive of toxicity on all doses used in acute toxicity testing. The LD_{50} of the aqueous extract of *Carpobrotus edulis* was estimated to be above 2000mg/kg. On subacute toxicity testing, there were no significance differences ($P<0.05$) on body weight changes, feed and water intake in all experimental groups. The serum biochemical results also did not show any significant variation among all the experimental groups. Gross pathology and histopathology examination of the selected organ tissues revealed no differences between control and treated Sprague Dawley Rats. It is concluded from the study that the aqueous extracts of *Carpobrotus edulis* are potentially safe.

Keywords: *Carpobrotus edulis*, Hepatotoxicity, Nephrotoxicity, Zimbabwe.

INTRODUCTION

Carpobrotus edulis (L.) N.E.Br. (Aizoaceae) also known as 'hottentot fig' or 'sea fig' ('*Igcukuma'* in IsiNdebele), is a very important traditional medicinal herb in Southern Africa since it is used for the treatment of various ailments. According to Steenkamp *et al*. [1] the leaves and flowers of *Carpobrotus edulis* are important in making decoctions used to treat bacterial and fungal infections. The leaves have an acerbic juice which is used as mouth gags for sore throat and mouth infections. Apart from antimicrobial activity, *Carpobrotus edulis* is also important in the management of diabetes mellitus and hypertension [2]. Pharmacological validations on the antimicrobial activity of *Carpobrotus edulis* has been studied extensively. Chokoe *et al*. [3] evaluated the antibacterial activity of ethanolic extracts of *Carpobrotus edulis* and the results showed significant activity against *staphylococcus aureus, Bacillus cereus, S* and *Mycobacerium aurum.* Phytochemistry studies from different parts of *Carpobrotus edulis* have revealed the presence of flavonoids, phenols, anthraquinones, saponins, cardiac glycosides, alkaloids and tannins [4, 5]. These phytochemical constituents are responsible for the purported medicinal properties of *Carpobrotus edulis*. Some phytochemicals however have been shown to have deleterious effects on human health [6]. It is therefore of paramount importance to perform a toxicological safety investigation of the aqueous extracts of *Carpobrotus edulis.*

The liver and kidneys are part of the important organs whose function should be closely monitored. Certain medicinal plants have proved to be hepatotoxic at higher doses [7]. The kidney receives 25% of the cardiac output and therefore it is very susceptible to intoxication [8]. Liver and kidney function tests are an important diagnostic tool whose biochemical parameters are necessary in detection of hepatotoxicity and nephrotoxicity respectively [9, 10]. Consumption of traditional medicines are on the increase as people prefer natural remedies to synthetic medicines found in orthodox treatment institutions [11]. However, caution should be taken when consuming plant extracts because they could have harmful effects on the body. Despite the extensive research in the medicinal properties of *Carpobrotus edulis*, there is paucity of

information regarding the toxicological profile of its extracts. The objective of this study was to evaluate the acute and subacute toxicity effects of aqueous extracts of *Carpobrotus edulis* in Sprague Dawley rats.

MATERIALS AND METHODS

The experimental protocols, care and handling of laboratory animals used in this study were in accordance with international guidelines (ARRIVE guidelines) [12]. All the experimental protocols, use and care of laboratory animals were approved by the Animal Research Ethics and Animal Welfare Subcommittee of the Division of Veterinary Services, Zimbabwe.

Plant collection and preparation of the extract

Leaves of *Carpobrotus edulis* were harvested, authenticated by an expert botanist, and then dried. The aqueous extract was prepared as described in our previous publication [5].

Laboratory animals and housing conditions

Healthy six-week-old Sprague Dawley rats (100-150 grams) of both sexes were obtained from the University of Zimbabwe animal house. The animals were kept in the Animal holding facility at the Department of Paraclinical Veterinary Studies, University of Zimbabwe. The rats were acclimatized to the laboratory conditions for a week prior to dosing. They were caged randomly in eight groups of four animals of each sex per group. The holding facilities were maintained under standard environmental conditions of 12 hours light and 12 hours darkness at temperature of 24ºC (±3ºC). The rats were allowed free access to food and water.

Acute toxicity

Three healthy female Sprague Dawley rats were used per step at any of the four fixed dose levels of 300, 600, 1200 and 2000mg/Kg body weight. A starting dose level of 300mg/kg was selected in accordance to the OECD test guideline 423 [13]. Food was withheld overnight but water was provided *ad libitum*. The Sprague Dawley rats were weighed using the electronic compact scale (SF-400A) just before extract administration through oral gavage. Food was withheld for a further 3-4 hours after the extract was administered. Clinical observations were made for signs of toxicity focusing on respiratory, circulatory, autonomic nervous system, central nervous system, changes in mucous membranes, skin and fur, eyes, behavioral pattern and death. Animals were kept for 14 days and were weighed weekly. On the 14th day, the animals were euthanized using hexane and gross necropsy was performed on all animals.

Subacute toxicity

The 28-day sub-acute toxicity study protocol was carried out as per the OECD number 408 guideline using 32 Sprague Dawley rats (16 males and 16 females) per step at any of the defined dose levels [14]. These rats were randomly allocated into four groups of eight rats (four females and four males). Group A received 100mg/kg of the extract while Group B and Group C received 300 mg/kg and 1000 mg/kg of the extract respectively. Group D served as a normal control and rats received only distilled water. All the experimental animals in all the groups received the extract orally for 28 days.

Body weight, food and water consumption

The rats were weighed prior to dosing at weekly intervals and the weight of each rat was recorded separately. Feed and water consumption were also calculated on a weekly basis.

Biochemical analysis

After treatment period, the rats were fasted overnight and put under general anesthesia with hexane. Blood was collected from all animals through cardiac puncture and was stored in plain blood collection tubes. The blood was then centrifuged using a Hermle Centrifuge (Hermle Z206A) at 3000 revolutions per minute in order to obtain serum. The serum obtained was put in Eppendorf tubes and stored at -20^{0}C while waiting biochemical analysis. Serum was also analysed at the Clinical Studies Department, University of Zimbabwe for Biochemical parameters using a Mindray Chemical analyser (BS 120). The determined parameters included Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Albumin, Total Protein, Urea and Creatinine.

Gross Pathology and Histopathology Examination

The animals were then euthanized humanely using hexane and subjected to post-mortem examination. Internal organs were examined for gross pathological changes. The organ weights of the heart, liver and kidney were taken for every experimental animal. After taking organ weights, these organs were fixed in 10% buffered formalin and submitted for histopathological processing. These organs were processed for histopathology through standard protocols. They were trimmed, embedded in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin and observed under the light microscope at 10x and 40x objective magnification.

Statistical analysis

Data analysis was done using Statistical package for Social sciences (SPSS version 21). The mean ± Standard error of the mean (SEM) values were calculated for each group. One- way analysis of variance (ANOVA) was used to compare mean biochemical differences within treatment groups. A level of $p<0.05$ was considered statistically significant.

RESULTS

Acute toxicity

There were no mortalities observed during the acute oral toxicity testing of *Carpobrotus edulis* aqueous leaf extract at a dose of 2000mg/kg. The LD_{50} of *Carpobrotus edulis* aqueous leaf extract from this study was estimated to be above 2000mg/kg. The animals in all the four treatment groups did not show any notable clinical signs. Table 1 shows the effects of *Carpobrotus edulis* on weekly mean body weight of Sprague Dawley rats after a single oral exposure.

Table 1: Weekly mean body weights of Sprague Dawley rat groups used in acute toxicity study of *Carpobrotus edulis* aqueous leaf extract

Group (n=3)	Dose levels (mg/kg)	weight in grams prior to *C. edulis* extract exposure	Weight in grams, one week after exposure	Weight in grams, two weeks after exposure
A	300	121.33 ±10.35	124.00 ±10.06	128.67 ±11.72
B	600	106.33 ±5.90	112.67 ±6.17	127.67 ±8.35
C	1200	131.33 ±19.47	132.00 ±18.77	136.67 ±19.27
D	2000	108.00 ±23.80	116.33 ±20.09	123.33 ±18.48

Values are expressed as means± SEM. *p value less than 0.05, ($p< 0.05$) significant value.

Subacute toxicity

Body weight, food and water consumption

There was no significant difference in body weight between experimental groups, even in weight gained. The percentage weight increases per treatment group are shown in Figure 1. Group C had an overall high weight increase compared to all the treatment groups while Group B had the lowest.

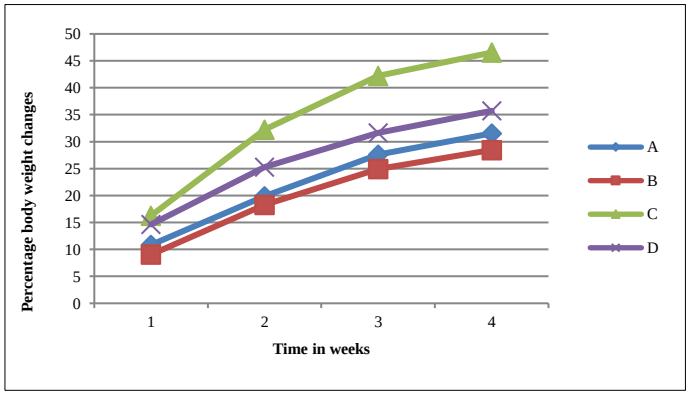

Figure 1: Percentage Body weight changes in Sprague Dawley rats treated with *Carpobrotus edulis* leaf aqueous extract.

The average daily water and feed intake of Sprague Dawley rats are shown in Table 2 and Table 3. There were no significant differences in water and feed intake between the treatment groups. Group D (control group) however had consistently higher water and feed intake compared to other treatment groups even though the differences were not statistically significant.

Table 2: Effect of *Carpobrotus edulis* on average daily water intake of Sprague Dawley Rats

Group (n=8)	Dose level (mg/kg)	Average water intake (ml)			
		Week 1	Week 2	Week 3	Week 4
A	100	95.00±1.94	97.50±4.40	115.14±9.37	98.36±4.24
B	300	96.93±3.31	101.00±5.44	110.43±9.55	107.64±3.78
C	1000	107.79±5.11	107.36±6.59	125.21±10.85	115.64±5.33
D	0	101.79±9.05	131.57±5.24	127.14±9.45	114.64±4.04

Values are expressed as mean± SEM. *p value less than 0.05, (p< 0.05) significant value.

Table 3: Effect of *Carpobrotus edulis* on Daily feed intake of Sprague Dawley rats

Group (n=8)	Dose level (mg/kg)	Average feed intake (g)			
		Week 1	Week 2	Week 3	Week 4
A	100	63.86±5.08	70.57±2.50	79.29±3.02	73.79±2.94
B	300	65.00±5.36	70.14±2.74	80.00±3.12	73.93±2.85
C	1000	67.79±5.56	75.43±2.19	80.64±4.26	75.64±3.09
D	0	71.71±6.33	80.71±3.21	86.07±2.76	80.07±3.42

Values are expressed as mean± SEM. *p value less than 0.05, (p<0.05) significant value.

Biochemical analysis

The clinical biochemistry results are summarized in Table 4. There were no significant variations (p>0.05) on all the measured biochemical parameters between all the treatment groups.

Table 4: Effect of aqueous extract of *Carpobrotus edulis* leaves on biochemical parameters in Sprague Dawley rats after subacute exposure

Biochemical indices	Treatment Groups			
	Group A (100mg/kg)	Group B (300mg/kg)	Group C (1000mg/kg)	Group D (0mg/kg)
Total protein (g/L)	59.63 ±1.20	60.15 ±1.50	54.23 ±5.22	51.36 ±4.31
Albumin (g/L)	35.35 ±0.34	41.70 ±6.38	33.26 ±1.60	33.51 ±1.80
ALT (IU/L)	57.93 ±4.81	51.95 ±5.64	58.53 ±2.31	52.44 ±2.86
ALP (IU/L)	136.00 ±7.12	139.28 ±11.56	135.85 ±35.61	132.43 ±20.14
AST (IU/L)	147.65 ±9.48	169.10 ±22.54	179.96 ±71.63	136.40 ±20.10
Urea (mmols/L)	9.50 ±0.43	7.90 ±0.74	9.11 ±1.01	10.11 ±0.78
Creatinine (µmol/L)	180.30 ±8.11	150.33 ±14.18	170.83 ±18.42	190.55±14.22
Total bilirubin (µmol/L)	8.48 ±2.28	12.86 ±4.30	10.79 ±1.65	15.71 ±5.33
Direct bilirubin(µmol/L)	7.23 ±1.93	9.69 ±2.90	9.49 ±1.55	11.90 ±3.94

Values are expressed as mean± SEM. *p value less than 0.05, (p<0.05) significant

Gross Pathology and Histopathology findings

There were no visible gross pathological lesions seen on all the animals. Organ weights of the heart, liver and kidneys were taken and their mean absolute weights are shown in Table 5. The aqueous extracts of *Carpobrotus edulis* did not show any abnormal effects on the histology of the liver, kidney and the heart. The photomicrographs of the liver, Kidney and the heart displayed normal histoarchitecture of these organs.

Table 5: Absolute organ weights of Sprague Dawley rats after 28 day repeated exposure to *Carpobrotus edulis* aqueous leaf extract.

Group (n=8)	Dose (mg/kg)	Organ weights in grams		
		Liver	Heart	Kidneys
A	100	8.328 ±0.537	1.571 ±0.127	1.703 ±0.148
B	300	8.451 ±0.584	0.982 ±0.081	1.711 ±0.142
C	1000	8.975 ±0.567	1.081 ±0.050	1.942 ±0.824
D	0	9.505 ±0.565	1.118 ±0.057	2.028 ±0.076

Values expressed as mean± SEM. *p value less than 0.05, (p<0.05) significant value.

DISCUSSION

Carpobrotus edulis is extensively used in traditional medicine in Southern Africa to treat various ailments. The phytochemicals found in *Carpobrotus edulis* extracts may however have harmful effects to biological systems. Safety assessment of the extracts of *Carpobrotus edulis* is therefore valuable in order to reduce a possible toxicological hazard to the exposed population. Acute toxicity study evaluates the toxicological effects of an extract or a drug due to a single exposure. A 14 day acute toxicity study of aqueous extract of *Carpobrotus edulis* was performed in Sprague Dawley rats and it showed no toxicological evidence at 2000mg/kg. According to this study, the aqueous extracts of *Carpobrotus edulis* can be classified as non-toxic due to absence of mortalities or any toxic clinical evidence observed at the dose of 2000mg/kg [13].

Body weight is an important indicator of toxicological effects of extracts or drugs [15]. Rapid bodyweight loss of about 15% to 30% within a week provides significant evidence of deleterious physiological effects of the extract or drug [16]. *Carpobrotus edulis* aqueous extract did not affect the body weight gains in relation to the control group in subacute studies. None of the experimental groups lost weight or gained more weight which could be attributed to the aqueous extract of *Carpobrotus edulis* treatment. The body weight of all the experimental animals followed a normal general trend. Feed and water intake of experimental animals are monitored in toxicological studies because this data gives an insight on the effect of the extract on the physiology and metabolism of these experimental animals. The differences in feed and water consumption of Sprague Dawley rats in this study in the treatment groups were not statistically significant (P<0.05) compared to the control group.

Biochemical parameters are key markers in toxicological evaluation as they give information on the deleterious effects of the extract to the liver or kidney [17]. The liver and the kidney are the primary organs prone to toxic effects of extracts or drugs [18]. Hepatic function tests and renal function tests are important indicators of toxicity which may not be clinically overt. Plasma urea measurements are key markers of acute kidney function. The rise in plasma urea is usually seen in acute and chronic kidney disease [19]. ALT is a specific marker to hepatic damage since only hepatocytes release ALT when damaged [20]. ALT, AST and ALP were measured in order to assess any hepatotoxicity while plasma urea and plasma creatinine levels were measured in order to evaluate the level of nephrotoxicity. Aqueous extracts of *Carpobrotus edulis* did not show any statistical difference (p<0.05) in the hepatic and renal function test in all experimental groups. All the biochemical parameters measured were within the normal reference ranges. This therefore shows that *Carpobrotus edulis* aqueous extracts do not possess any hepatotoxicity and nephrotoxicity effects.

Gross pathological changes are important indicators of tissue damages in living organisms [21]. On gross pathological examination, the kidney and liver of Sprague Dawley rats in the treated groups did not show any changes in shape, colour, size and texture when compared to the control group of rats. Relative organ weight is also a significant indicator of physiological status [21]. The liver, kidney, lung and spleen are the primary target organs by toxicants and/or their metabolites [22]. The liver, kidney and heart relative organ weights were evaluated in this study. Aqueous extracts of *Carpobrotus edulis* did not show any significant effects on relative organ weights in all the experimental groups. An astounding change in relative organ weight among treated and untreated experimental animals is a marker of toxicity as organ weight is influenced by the suppression of body weight. Toxic phytochemicals might result in cellular degeneration and necropsy of body organs. The liver, kidney and heart of Sprague Dawley rats were evaluated for any histopathologic changes. Histology remains the gold standard diagnostic tool for structural related organ damage [23]. The general histoarchitecture of all the evaluated organs in treated groups did not show any significant differences when compared to the control group. The histopathology results concur with the biochemical results which showed no adverse effects when Sprague Dawley rats were treated with aqueous extracts of *Carpobrotus edulis.*

CONCLUSION

The acute toxicity study of aqueous extract of *Carpobrotus edulis* did not produce deleterious effects on the behavior of female Sprague

Dawley rats. The oral LD_{50} of the *Carpobrotus edulis* aqueous extract was found to be above 2000mg/kg and therefore classified as less toxic. In the subacute toxicity study, there were no adverse effects observed on bodyweight, feed/water intake, histology or on the tested biochemical parameters of Sprague Dawley rats. It is concluded that the phytochemicals in aqueous leaf extracts of *Carpobrotus edulis* do not have harmful effects at 1000 mg/kg in Sprague Dawley rats. Clinical toxicity evaluations of *Carpobrotus edulis* extracts in humans are recommended to ascertain safe doses since toxicity may not be entirely extrapolated animal studies.

Declaration of conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship and publication of this article.

Acknowledgements

We are very grateful to the Faculty of Veterinary Science, University of Zimbabwe for their facilities and equipment used in this project. The authors wish to thank Mr. Isaiah Mujakwi for taking care of the laboratory animals during the study.

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HOW TO CITE THIS ARTICLE
Mudimba TN, Mbaria J, Maitho T, Taderera T. Acute and subacute toxicity evaluation of aqueous extracts of *Carpobrotus edulis* in Sprague Dawley rats. J Phytopharmacol 2020; 9(4):265-269.