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Hematotoxicity assessment of phytochemicals from aqueous leaf extracts of *Carpobrotus edulis*

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ABSTRACT

The leaves of *Carpobrotus edulis* are used in folk medicine to treat several ailments. This study therefore seeks to do a phytochemical screening of the aqueous leaf extract of *Carpobrotus edulis* which grows in Zimbabwe as well as to determine the effect of these aqueous extracts on hematological parameters of Sprague Dawley rats. Aqueous extraction of *Carpobrotus edulis* leaves was performed by using routine methods and standard phytochemical screening methods. Thirty-two (32) Sprague Dawley rats of both sexes equally represented, were grouped into three treatment groups (A-C) and one negative control group D of eight animals each. Group A received 100mg/kg of the extract; Group B received 300mg/kg while Group C received 1000mg/kg of the extract for 28 days. After 28 days of daily oral gavage of the extract, hematological parameters were measured. Phytochemical screening revealed the presence of flavonoids, anthraquinones, alkaloids, terpenoids, saponins, tannins and glycosides. Aqueous leaf extracts of *Carpobrotus edulis* did not have any significant effects ($P>0.05$) on the hematological parameters on any of the treatment groups of Sprague Dawley rats after oral exposure for 28 days. *Carpobrotus edulis* have no deleterious effects on the hematological parameters however further studies are recommended to completely evaluate the safety profile of the plant.

Keywords: *Carpobrotus edulis*, Phytochemistry, Hematological effects, Zimbabwe.

INTRODUCTION

Blood is a vital body fluid which consists of blood cells and blood plasma. Blood cells constitute about 45% of the blood while 55% of blood is plasma [1]. These blood cells consist of erythrocytes, leukocytes and thrombocytes. Erythrocytes are anucleate blood cells constituting about 45% of all blood which contains hemoglobin which is important for distribution of oxygen to the various body cells and taking carbon dioxide from the same body cells [2]. Leukocytes are also important part of the body's immune system responsible for destroying infectious agents and removal of aberrant cellular debris and any other foreign substances. Thrombocytes also known as platelets are important in blood coagulation process. These blood cells are vital hematological parameters which are useful for normal body function and act as an important diagnostic tool [3].

Deviation from normal ranges of these hematological parameters can be caused by various causes and can have harmful consequences [4]. Certain phytochemical groups such as saponins and flavonoids have been known to alter the hematological parameters of animals [5]. *Carpobrotus edulis* which grows in Zimbabwe potentially contains various phytochemicals that may have an effect on these hematological parameters.

Carpobrotus edulis is commonly known as *Igcukuma* by Xhosa and Ndebele communities of Zimbabwe and is an edible ground cover plant which is used widely to treat hypertension in Southern Africa [6]. The plant is native in South Africa and is commonly known as ice plant, Hottentot-fig or sour fig. *Carpobrotus edulis*, a plant in the genus *Carpobrotus* and member of the *Aizoaceae* family, is a perennial creeping subshrub with mat-forming succulent leaves and low-lying stems that may reach several meters [7]. It is commonly used by the traditional therapist in the Eastern Cape Province in South Africa in the treatment of diabetes mellitus, tuberculosis, sores, constipation and intestinal worms [8].

Carpobrotus edulis is one of the commonest plants used in traditional medicine in Zimbabwe which contains various phytochemicals and their effects on hematological parameters remain unknown. This study therefore seeks to do a phytochemical screening of the aqueous leaf extract of *Carpobrotus edulis* which grows in Zimbabwe as well as to determine the effect of these aqueous extract on hematological parameters of Sprague Dawley rats.

MATERIALS AND METHODS

Plant Collection and preparation of the extract

Carpobrotus edulis plant leaves were collected from Cannock Gardens in Mount Pleasant area of Harare, Zimbabwe. The plant was authenticated by a Botanist from the National Herbarium and Botanical Gardens of Zimbabwe, and a voucher specimen (Mudimba, T.1 08/01/2019) was properly marked and stored.

The leaves were first washed free of sand and debris. The leaves were air dried at room temperature (24°C ±3°C) for 12 days and ground into powder. The powder weighing 300g was macerated in distilled water at a ratio of 1 to 6 (w/v) in a volumetric flask. The suspension was macerated for 48 hours at room temperature with constant shaking. The suspension was then filtered using Whatman® filter paper (number 4). The filtrate was freeze dried (Edwards-Freezer Dryer Modulyo, EF4) for 48 hours and the extract was weighed in order to determine extract yield.

Phytochemical screening

Qualitative phytochemical screening methods were used in order to identify the phytochemical constituents of aqueous leaf extracts of *Carpobrotus edulis*. The standard qualitative methods used by Ajuru *et al* [9] were used in order to detect the presence or absence of flavonoids, anthraquinones, alkaloids, terpenoids, saponins, tannins and glycosides.

Experimental Animals and experimental protocol

Before commencement of the study, ethical approval (Ref number 001/2019) on the ethical use and care of laboratory animals was obtained from the Animal Research Ethics and Animal Welfare Sub-Committee of the Division of Veterinary Services, Zimbabwe. The rats were disposed according to the guidelines given by the ethical Committee from the Division of Veterinary Services in Zimbabwe.

Thirty-two Sprague Dawley rats (150-200g) of both sexes were obtained from the University of Zimbabwe Animal House and were housed in the animal holding facilities at the Faculty of Veterinary Science, University of Zimbabwe. They were caged in pairs and maintained under standard environmental conditions of 12 hours light and 12 hours darkness at 24°C (±3°C). The rats were fed on commercial rat pellets obtained from the Zimbabwe National foods. Drinking water was provided to the rats ad libitum from tap water.

These Sprague Dawley rats were randomly allocated into four groups of eight rats (four females and four males). Group 1 received 100mg/kg of the extract while Group 2 and Group 3 received 300 mg/kg and 1000 mg/kg of the extract respectively. Group 4 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.

Collection and handling of blood samples

After treatment period, animals were fasted overnight and were put under general anaesthesia using halothane. Blood was collected from all animals through cardiac puncture. The collected blood was put in

blood collection tubes which contained Ethylenediaminetetraacetic acid (EDTA).

Determination of Hematological parameters

Blood collected in EDTA containing tubes was analysed in the Clinical Studies Department of the University of Zimbabwe by using a Mindray Haematology analyser (BC2800 vet). The parameters which were determined included Total Red Blood Cell count (RBC), Red Blood Cell Distribution Width (RDW), Total Leucocyte Count (WBC), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet counts and Mean Platelet Volume (MPV).

Statistical analysis

Data was captured on Microsoft Excel and transferred to Statistical Package for Social Sciences software (SPSS @version 21.0) for further analysis of the data. Hematological parameters were expressed as means ± standard error of the mean (SEM) for all the groups. One-way ANOVA was used to compare the variation of hematological profiles within groups and a 95% level of significance (p ≤0.05) was used in the analysis.

RESULTS

Phytochemical composition of aqueous leaf extracts of *Carpobrotus edulis*.

Eight phytochemical groups were found to be present in the aqueous extracts of *Carpobrotus edulis* as shown in Table 1.

Table 1: Phytochemical Composition of aqueous extracts of *Carpobrotus edulis* leaves.

Phytochemical group	Presence/absence in <i>Carpobrotus edulis</i> aqueous leaf extract
Phenols	++
Flavonoids	+
Anthraquinones	+++
Alkaloids	+
Terpenoids	+
Saponins	++
Tannins	+++
Glycosides	++

Highly present+++; moderately present ++; lowly present +

Hematological analysis

The effect of *Carpobrotus edulis* aqueous extract on erythrocyte indices in Sprague Dawley rats after a 28-day repeated oral exposure are shown in Table 2. There were no significant differences in all measured Hematological parameters between the experimental groups of Sprague Dawley rats after 28 day-repeated oral exposure to aqueous *Carpobrotus edulis* extracts.

Table 2: Effects of the *Carpobrotus edulis* aqueous leaf extract on Hematological indices in Sprague Dawley rats after a 28-day repeated oral exposure.

Hematology indices	Treatment Groups			
	Group A (100mg/kg)	Group B (300mg/kg)	Group C (1000mg/kg)	Group D (0mg/kg)
RBC ($\times 10^6$ cells. μL^{-1})	9.21 \pm 0.30	9.18 \pm 0.20	9.23 \pm 0.22	9.45 \pm 0.21
Hb (g.dL ⁻¹)	17.21 \pm 0.32	18.06 \pm 0.38	18.45 \pm 0.31	18.61 \pm 0.30
Hct (%)	53.48 \pm 1.07	52.78 \pm 1.13	53.45 \pm 1.09	54.45 \pm 1.09
MCV (fL)	58.46 \pm 1.06	57.45 \pm 0.72	57.87 \pm 0.62	57.62 \pm 0.41
MCH (pg.cell ⁻¹)	19.71 \pm 0.28	19.58 \pm 0.25	19.71 \pm 0.40	19.87 \pm 0.22
MCHC (g.dL ⁻¹)	33.91 \pm 0.22	34.15 \pm 0.15	34.52 \pm 0.29	34.21 \pm 0.23
RDW (%)	15.09 \pm 0.57	14.46 \pm 0.24	13.93 \pm 0.61	14.60 \pm 0.30
WBC ($\times 10^3$ cells. μL^{-1})	6.96 \pm 0.97	5.93 \pm 1.22	7.55 \pm 1.79	7.42 \pm 1.16
Platelets ($\times 10^3$ cells. μL^{-1})	534.00 \pm 30.65	639.25 \pm 47.93	608.25 \pm 29.62	576.88 \pm 28.75
MPV (fL)	5.35 \pm 0.78	5.29 \pm 0.68	5.36 \pm 0.92	5.48 \pm 0.14

Values are expressed as mean \pm SEM, n=8 *p value less than 0.05, (p< 0.05) significant value

DISCUSSION

The phytochemical screening results indicated presence of crucial secondary plant metabolites such as flavonoids, alkaloids, tannins, phenols, terpenoids, saponins, anthraquinones and glycosides. These results are in accordance with the study done by Eman, [10] who investigated the phytochemical constituents of succulent plants found in Egypt. Phytochemicals usually have a potential of medicinal properties and often serve as leads to the development of new drugs [11]. The presence of these phytochemicals partly justifies the common herbal use of *Carpobrotus edulis*. The detailed effects of these phytochemicals in normal body physiology are however subject to further investigation since some may have harmful effects in the body. Extracts of *Allium sativa*, for example increased osmotic fragility of red blood cells in Wistar rats [12]. The aqueous extracts of *Carpobrotus edulis* however did not show any harmful hematological effects in Sprague Dawley rats. The evaluation of hematological parameters is very useful in determination of deleterious effects which may be caused by medicinal plants [11]. Hematological parameters are essential diagnostic tools and such evaluations have higher predictive value on the toxicity of medicinal plants [13]. Hematological evaluation carried out for aqueous extracts of *Carpobrotus edulis* on Sprague Dawley rats did not show any significant differences between different treatment groups. White blood cells are responsible for the first response to infectious agents and/ or any tissue injury. Saponins from different medicinal plants are known to affect negatively white blood cell function [14]. In this study, saponins in the aqueous extracts of *Carpobrotus edulis* did not affect the white blood cell parameters suggesting that the normal physiological mechanisms that maintain white blood cells were not altered.

The insignificant effect of aqueous leaf extract on RBC, MCV, MCH, MCHC, Platelets and MPV show that *Carpobrotus edulis* does not affect the production and/ or the destruction of erythrocytes and thrombocytes. The presence of alkaloids have been associated with the reduction in RBC count [15] however in this study, alkaloids from aqueous extracts of *Carpobrotus edulis* leaves did not have significant effects on red blood cells.

Effects on thrombocyte parameters can have harmful consequences since thrombocytes are essential for hemostasis. Some phytochemicals are potential therapeutic agents for thrombocytopenia [16]. This was discovered through studying the effects of different extracts on platelets. Phytochemicals in the aqueous extracts of *Carpobrotus edulis* leaves did not affect thrombocytes in Sprague Dawley rats.

CONCLUSION

In conclusion, this study has shown that aqueous leaf extracts of *Carpobrotus edulis* which grows in Zimbabwe contain important phytochemicals. These phytochemicals have potential medicinal properties and that is why it is a common herbal medicinal plant in Southern Africa. It is concluded that the phytochemicals in aqueous leaf extracts of *Carpobrotus edulis* do not have harmful effects on hematological parameters of Sprague Dawley rats. Further toxicity studies on *Carpobrotus edulis* extracts are recommended in order to ascertain completely the safety profile of the medicinal plant.

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Declaration of conflict of interest

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

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