British Journal of Pharmaceutical Research
15(6): 1-10, 2017; Article no.BJPR.32346
ISSN: 2231-2919, NLM ID: 101631759

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Haematological and Biochemical Properties of Methanolic Extract of *Citrullus lanatus* Seeds

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GTA, AO and OB designed the study, performed the statistical analysis, and wrote the protocol. Author GTA wrote the first draft of the manuscript. Authors GTA, OB and AO managed the analyses of the study. Authors GTA and AO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2017/32346
Editor(s):
(1) Salvatore Chirumbolo, Clinical Biochemist, Department of Medicine, University of Verona, Italy.
Reviewers:
(1) Jana Sic Zlabur, University of Zagreb, Croatia.
(2) Wan Mazlina Md Saad, Universiti Teknologi MARA, Malaysia.
(3) Simon Bernard Iloki Assanga, Laboratorio de Investigación en Bioactivos y Alimentos Funcionales (LIBAF), RubioPharma y Asociados SA de CV, Mexico.
Complete Peer review History: http://www.sciencedomain.org/review-history/18722

Original Research Article

Received 22nd February 2017
Accepted 27th March 2017
Published 21st April 2017

ABSTRACT

Watermelon is a commonly consumed, but this consumption is usually limited to the fruit. Phytochemical constituents of watermelon have known advantages, however the seeds of the plant are usually thrown away. This study investigated the possible beneficial effects of consumption of watermelon seeds, assessing their effects on blood, liver and general metabolism. Twenty-four (24) male rats were divided into four (4) groups consisting of six (6) rats each and were treated for 20 days. Group 1 (control) rats received normal saline, group 2 were given 100mg/kg of the *Citrullus lanatus* extract, while group 3 and 4 received 200 and 300 mg/kg of the *Citrullus lanatus* extract respectively. Blood samples were collected after 10 days and 20 days for analysis. Weekly body weights were taken, while red blood cell and platelet counts were determined. Packed cell volume, haemoglobin concentration and white blood cell differential count were also determined using standard methods. Alanine transaminase (ALT), Aspartate transaminase (AST), Alkanine phosphatase (ALP) and bilirubin concentrations were also estimated from serum. There was a significant decrease ($p<0.05$) in the serum levels of ALT, AST and

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bilirubin in groups that received the seed extract while there were significant increases in haematological indices. There was also a significant decrease in body weight of the rats treated with the extract in individual comparisons with the control group on days 10 and 20 of administration. Consumption of *Citrullus lanatus* seed extract reduces body weight, decreases serum levels of liver biomarkers and increases haematological parameters. Hence, *Citrullus lanatus* seed is safe for human and might be beneficial effect on health.

Keywords: Watermelon; Citrullus lanatus; blood; liver enzymes; haemotological parameters; biochemical variables.

1. INTRODUCTION

Citrullus lanatus (watermelon) belongs to the *Cucurbitaceae* family [1] and is one of the most popular of the species. It has high water content (up to 92% of total weight) [2] and is a commonly consumed fruit worldwide. The nutritional value and phytochemical content of many fruits, rind, especially the seeds, has not been given much attention, such that most times these parts of fruit are discarded even with their hidden nutrients. Interestingly, the seed and rind of some fruits including that of *Citrullus lanatus* have higher vitamins, fibres, minerals and other essential nutrients activity than the pulp fractions [3]. The *Citrullus lanatus* fruit is used in cooling, strengthening, as an aphrodisiac, and is astringent to the bowels. It has also been reported to allay thirst, cure biliousness, sore eyes, scabies and itches and serve as a brain tonic [4]. *Citrullus lanatus* seed contains phytochemical constituents like alkaloids, flavanoids, tannins, amino acids, carbohydrates, cardioglycosides, terpenoids, oils and fats in the methanolic extract of plant material when compared with other solvents, however, these seeds are often thrown away without considering their potential nutritional and medicinal benefits [5]. The flavanoids they contain, for example, have been reported to possess antiviral, anti-allergic, anti-platelet, anti-tumor and antioxidant activities [6]. Also, the antibacterial [7], hepatoprotective [8], anti-inflammatory [9], anti-ulcerative [10], and anti-microbial [11] effects of the seeds of the *Citrullus lanatus* plant have been investigated and elucidated upon in literature.

Disorders of metabolism such as metabolic syndrome, and its main precursor obesity, have become a problem of epidemic proportions in both the developed, and more recently developing societies. More than two-thirds of the adult population of the United States has been described as overweight, with a third of the population being obese [12]. Interestingly and most importantly, the fastest growth rates of obesity have been reported in children [13,14]. This epidemic is being perpetuated by increasing consumption of calorically-dense but highly-palatable foods [15]. A theory of endocrine disruption postulates that exogenous chemicals can modulate homeostasis by interfering with the action of endogenous hormonal axes [16]. This has led to an “environmental obesogen hypothesis”, which postulated a causal link between “obesogens” in the environment and the obesity epidemic [17]. A major environmental factor influencing metabolism is the diet, or specific dietary macro and micro nutrients in the diet consumed by an individual. As such, diet is implicated in the pathways involved in the generation of insulin resistance leading to Type II diabetes mellitus and metabolic syndrome, via its role in inflammation and effects on the liver. However, these exogenous chemicals from diet can also have a positive effect on, and possibly reverse obesity and its pathway to Type II diabetes mellitus and metabolic syndrome.

The liver on its own plays a major role in the metabolism of toxic substances that enter the body. Maintenance of the functional integrity of the liver is also essential to maintain normal metabolism and homeostasis of carbohydrates, lipids, and amino acids [18]. Serum biochemical parameters are important indicators which are used in monitoring physiologic and pathologic changes in the liver. The values of these parameters can be used for monitoring any changes in the health status of animals [19]. Combined with haematology, the biochemical profile forms the data base for most diagnostic investigations. Many biochemical parameters tend to have specificity for an organ and/or a limited range of pathological processes. Ferreira et al., [20] reported that biochemical parameters provide early warning of potentially harmful changes in stressed organisms.

Investigating the haematological parameters represents a useful process in the diagnosis of

many diseases as well as investigation of the extent of damage of blood [21]. This is relevant since blood constituents' change in relation to the physiological conditions of animals. Haematological studies are important because the blood transports or conveys nutrient and materials to different parts of the body. Therefore, whatever affects the blood; drugs, pathogenic organism or nutrition will certainly affect the entire body adversely or moderately in terms of health, growth, maintenance and reproduction [22,23]. Thus, evaluation of the haematological profile usually furnishes vital information on the body's response to injury of all forms. Haematological constituents also reflect the physiological responsiveness of the animal to its internal and external environments [24]. The examination of blood also provides the opportunity to clinically investigate the presence of several metabolites and other constituents in the body and it plays a vital role in the physiological, nutritional and pathological status of the animal [25,26].

Many studies suggest that endogenous antioxidants, or exogenous antioxidants supplied by diet, can function as free radical scavengers and improve human health [27,28,29,30]. Thus, consumption of a variety of plant foods including *Citrullus lanatus* seeds may provide additional health benefits. There have been several research works on the effects of *Citrullus lanatus* seeds on various variables in the body and in various disease conditions. Despite much excellent work on themes such as the healing power of *Citrullus lanatus* in acetic acid induced ulcerated rats [31], the effects on induced prostatic hyperplasia [32] and on induced liver damage [33]. However, none of these works have addressed the central question on its effects on haematological and biochemical variables or its possible metabolic effects.

2. MATERIALS AND METHODS

2.1 Plant Material

Citrullus lanatus seeds were bought from *Farin gada* market in Jos, Nigeria. They were identified and authenticated by a botanist in the Department of Botany, University of Ibadan, as *Citrullus lanatus* with authentication number UIH-22504. The seeds were then rinsed and oven-dried at 60°C for 90 minutes, after which they were subsequently air-dried for 1 week and finally ground into fine powder. The methanolic extract was prepared by soaking 2800 grams of the powdered sample in 3500ml of 80% methanol for 72 hours. The mixture was then filtered through cheese cloth and then filter paper. The filtrate obtained was evaporated, concentrated at room temperature and stored at 4°C in a freezing chamber.

2.2 Experimental Animals

Twenty-four (24) male Wistar rats, weighing between 150g and 180g, were bought from Olu Farms in Ibadan, Nigeria. The animals were housed in animal cages with suitable temperature and humidity in the Animal House of Department of Physiology, Bowen University, Iwo, Osun state, Nigeria and were acclimatized for 2weeks before the commencement of the experiment. They had free access to food and water throughout the period of the experiment and they were kept under standard laboratory conditions. Handling and use of animals in this study were in accordance with the guiding principles for research involving animals as recommended by the declaration of Helsinki and the Guiding principles in the care and use of animals [34].

2.3 Experimental Design

Twenty-four (24) male rats were divided into four (4) groups consisting of six (6) rats each and subjected to the following oral treatments for 20 days.

Group 1 (control): rats received normal saline.

Group 2: rats received 100mg/kg of the *Citrullus lanatus* extract.

Group 3: rats received 200mg/kg of the *Citrullus lanatus* extract.

Group 4: rats received 300mg/kg of the *Citrullus lanatus* extract.

Blood samples were collected after 10 days and 20 days for analysis.

2.4 Determination of Body Weight

The body weights of the rats were taken before the start of the experiment and at the end of each week throughout the period of the experiment. The measurements were taken using a digital Camry® sensitive weighing scale.

2.5 Collection of Blood for Analysis

Twenty four hours after the last dose of administration of the extract, the animals were anaesthetized with chloroform and sacrificed. Blood was obtained via cardiac puncture into plain sample tubes and allowed to stand for 2 hours and thereafter centrifuged at 2000rpm for 15 minutes to separate serum from the blood cells.

2.6 Haematological Analysis

The procedures for haematological variables were carried out at Lab Masters, a medical laboratory in Ibadan, Nigeria. The variables were analyzed using a Mindray® BC-2800 auto haematology analyzer which is a quantitative, automated analyzer and leucocyte differential counter.

2.7 Serum Analysis

Blood was collected from the rats by cardiac puncture after anaesthesia with Chloroform. The blood was left to clot in plain bottles after which it was centrifuged at 5000 rpm for 5 minutes and then the serum was collected into plain serum bottles. The serum was used to estimate Alanine transaminase (ALT), Aspartate transaminase (AST), Alkanine phosphatase (ALP) and bilirubin concentrations which are all tests of liver function. Activities of ALT and AST were assayed by the method of Reitman and Frankel [35], while ALP activity was determined by Rec. GSCC method [36]. Bilirubin concentration determination was by 2,5-dichlorphenyldiazonium method described by Schlebusch et al. [37].

2.8 Statistical Analysis

Results were expressed as mean ± SEM (standard error of mean) and analysed using one-way ANOVA (analysis of variance) with Newman-Keul's multiple comparism (Post HOC) test using the Graph Pad Prism 6.0 software. All results were considered significant at $p < 0.05$.

3. RESULTS

3.1 Effect of *Citrullus lanatus* Seed Extract on Body Weight in Male Wistar Rats

A steady increase in weight was observed in the control group throughout the experiment. Rats in group 2 (187.8±1.62) and group 3 (169.6±2.91) showed significant decreases in body weight when compared to group 1 (201±4.96) at day 10 while rats in all treatment groups (180.6 ±0.68; 164.4±2.54; 179.6±3.87) showed significant decrease in their body weights when compared to the control (208±4.68) by day 20.

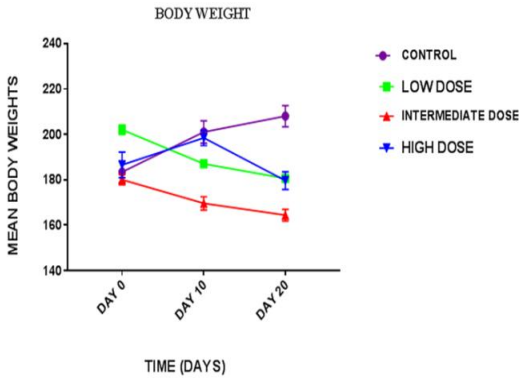

Fig. 1. Body Weight Changes in male rats in response to *Citrullus lanatus* over a period of 20 days

3.2 Effect of *Citrullus lanatus* Seed Extract on Different Haematological Variables

The results in Table 2 show that there were statistically significant increases ($P<0.05$) in packed cell volume in all the treatment groups (46.2± 0.58%, 43.2 ± 1.06%, 44 ± 0.7%) when compared individually with control (39.2 ± 1.24%), after 10days of administration. Only rats in group 2 (701.6±14.08) and 3 (719.4±15.78) showed a significant increase in platelet count after 10 days compared to the control (596.8±23.96). However, after 20 days of administration, Haemoglobin concentration was increased in group 2 (12.8± 0.68g/dL) and group 4 (12.98 ± 0.2 g/dL) only in individual comparison with the control (14.3±0.23 g/dL). Also, rats in group 3 ($8.44±0.11x10^6$/ µL) showed a significant increase in red blood cell count after 20 days of administration when compared to the control ($7.36±0.16x10^6$/ µL).

White blood cell count was decreased in group 2 ($9.82 ± 0.23 x 10^3$/µL) and there was also a significant decrease in neutrophil proportion in group 2 (23.6±0.75%) as well as group 4 (24.4 ±0.87%) when compared individually to the control (13.5 ± 1.27%; 31± 0.98%) after 10 days. Group 4 ($20.4 ±4.12 x 10^3$/µL) rats also reflected an increase in white blood cell count when

Table 1. Effects of *Citrullus lanatus* seeds extract on Packed Cell Volume (PCV), Haemoglobin concentration (Hb), Platelets count (PLT) and Red Blood Cell Count (RBC)

	Time (Days)	Group 1 (Control)	Group 2 (Low Dose 100mg/kg)	Group 3 (Intermediate dose 200mg/kg)	Group 4 (High Dose 300mg/kg)
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
PCV (%)	10	39.2 ± 1.24	46.2 ± 0.58*	43.20 ± 1.06*	44.0 ± 0.70*
	20	48.4 ± 1.33	45.2 ± 0.66	51.4 ± 2.25	44.6 ± 0.75
HB(g/d L)	10	13.4 ± 0.72	12.7 ± 0.46	12.2 ± 0.50	13.5 ± 0.40
	20	14.3 ± 0.23	12.8 ± 0.68*	14.4 ± 0.34	12.98 ± 0.20*
PLT	10	596.8 ± 23.96	701.6±14.08*	719.4 ± 15.78*	579.40 ± 16.20
	20	792.6 ± 28.5	574 ± 24.57*	858 ± 40.73	715.4 ± 15.51
RBC (10^6/ µ L)	10	6.7 ± 0.27	7.56 ± 0.13	7.58 ± 0.23	6.98 ± 0.32
	20	7.36 ± 0.16	7.58 ± 0.12	8.44 ± 0.11*	7.68 ± 0.13

Table 2. Effects of *Citrullus lanatus* seed extract on white blood cell count in the different experimental groups of rats (n=5)

	Time (Days)	Group 1 (Control)	Group 2 (Low dose 100 mg/Kg)	Group 3 (Intermediate dose 200 mg/Kg)	Group 4 (High dose 300 mg/Kg)
WBC(10^3/µ L)	10	13.5 ± 1.27	9.82 ± 0.23*	13.9 ± 0.65	13.52 ± 0.40
	20	10.2 ± 0.14	8.48 ± 0.5	11.7 ± 1.08	20.4 ± 4.12*
NEUT (%)	10	31.0 ± 0.98	23.6 ± 0.75*	30.8 ± 1.62	24.4 ± 0.87*
	20	26.0 ± 0.70	36.8± 1.74*	32.8 ± 0.97*	31.4 ± 2.01*
LYM (%)	10	70.00 ± 1.84	71.0 ± 3.58	65.8 ± 2.98	68.4 ± 1.21
	20	65.2 ± 2.47	60.6 ± 1.94	68.6 ± 1.16	73.6 ± 0.81*
MONO (%)	10	3.8 ± 0.66	2.8 ± 1.07	1.6 ± 1.10	4.4 ± 1.33
	20	1.8 ± 0.49	3.8 ± 0.66	1.6 ± 0.75	2.4 ± 0.75
EOSI (%)	10	1.2 ± 0.58	1.0 ± 0.45	0.8 ± 0.49	2.2 ± 0.66
	20	1.4 ± 0.58	0.8 ± 0.49	3.2 ± 1.62	1.8 ± 1.11

*Values expressed as mean ± SEM *p<0.05 compared to the control (n=6)*

compared to the control (10.2 ± 0.14 x 10^3/µL) after 20 days. Differential White blood cell analysis showed a significant increase in neutrophils count in group 2 (36.8±1.74%), group 3 (32.8±0.97%) and group 4 (31.4±2.01%) and also a significant increase in lymphocyte count in group 4 (73.6±0.81%) when compared individually to the control (26.0±0.70%; 65.2±2.47%), after 20 days of administration (Table 2).

The results for the effects of *Citrullus lanatus* Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) are as shown in Table 3. There was a significant increase in MCV in group 2 (62.6±0.87µm^3) compared to the control (59.6 ±0.93µm^3). No significant difference was recorded in values of mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration.

3.3 Effect of *Citrullus lanatus* Seed Extract on Biochemical Variables

3.3.1 Aspartate amino transferase (AST)

There was a significant decrease in the mean value for the level of AST in group 2 (246.6 ± 6.3), group 3 (260.8 ± 3.5) and group 4 (211±5.13) compared to group 1 (300.4 ± 3.3) after 10 days of administration. At the end of 20 days, there was also a significant decrease in the mean value for the level of AST in group 2 (273.4± 3.1) and group 3 (288.8 ±4.18) when compared to group 1 (336 ± 2.8).

Table 3. Effects of *Citrullus lanatus* seed extract on MCV, MCHC, MCH in the different experimental groups of rats (n=5)

	Time (Days)	Group 1 (Control)	Group 2 (Low Dose 100 mg/Kg)	Group 3 (Intermediate Dose 200 mg/Kg)	Group 4 (High Dose 300 mg/Kg)
MCV (μm^3)	10	59.6 ± 0.93	62.6 ± 0.87*	60.4 ± 1.17	58.8 ± 0.97
	20	58 ± 0.89	58.4 ± 1.69	59.8 ± 0.73	59.6 ± 0.93
MCH (pg/cell)	10	18.6 ± 0.67	17.6 ± 0.24	18.2 ± 0.58	17.4 ± 0.24
	20	17.4 ± 0.24	17.2 ± 0.49	17.4 ± 0.24	17.2 ± 0.37
MCHC (%)	10	29.4 ± 0.73	28.56 ± 0.18	28.86 ± 0.46	28.8 ± 0.76
	20	28.96 ± 0.65	29. 58 ± 0.10	29.12 ± 0.19	29.0 ± 0.21

*Values expressed as mean ± SEM *p<0.05 (n=6)*

Table 4. Effect of *Citrullus lanatus* seed extract on biochemical Variables

	Days	Group 1 (control)	Group 2 (low dose 100mg/kg)	Group 3 (intermediate dose 200mg/kg)	Group 4 (high dose 300mg/kg)
AST (U/L)	10	300.4 ± 3.3	246.6 ± 6.3*	260.8 ± 3.5*	211 ± 5.13*
	20	336.0 ± 2.8	273.4 ± 3.1*	288.8 ± 4.18*	232.8 ± 6.87
ALT (U/L)	10	43.0 ± 1.52	43.2 ± 2.2	40.2 ± 1.96	40.6 ± 3.41
	20	41.2 ± 1.83	40 ± 1.58*	42.4 ± 2.79*	34.4 ± 0.93
ALP (U/L)	10	214.8 ± 2.63	390.2 ± 13.9*	387.2 ± 5.99*	136.4 ± 5.89*
	20	167.8 ± 6.03	246.8 ± 3.62*	223.4 ± 6.12*	238.2 ± 4.47*
BILIRUBIN (mmol/L)	10	0.36 ± 0.04	0.20 ± 0.03	0.26 ± 0.07	0.42 ± 0.09
	20	0.34 ± 0.05	0.2 ± 0.03*	0.22 ± 0.02*	0.44 ± 0.02

*Values expressed as mean ± SEM *p<0.05 compared to the control (n=6)*

3.3.2 Alanine amino transferase (ALT)

There was a significant decrease in the mean value for the level of ALT in group 2 (40±1.58) while there was a significant increase in the mean value for group 3 (42.4 ± 2.7) compared to group 1 (41.2 ± 1.83) at the end of 20 days.

3.3.3 Alkaline phosphatase (ALP)

After 10 days, there was a significant increase in the mean value for the level of ALP in group 2 (390.2 ± 13.9) and group 3 (387.2 ± 5.99) but a decrease in Group 4 when compared to Group 1. However, group 2, 3 and 4 showed a significant increase in mean value of ALP after 20 days.

3.3.4 Bilirubin

There was a significant decrease in the mean value for the level of Bilirubin in group 2 (0.2 ± 0.03) and group 3 (0.22 ± 0.02) compared to group 1 (0.34 ± 0.05) at the end of 20 days but there was no significant difference at the end of 10 days.

4. DISCUSSION

The effect of methanolic extract of *Citrullus lanatus* seed on body weight, biochemical and haematological parameters was examined in this study.

The decrease (P<0.05) observed in body weight across all the groups treated with the extract suggests that *Citrullus lanatus* seed may have a significant role to play in weight loss. This is of importance in Type II diabetes mellitus, where the main predisposing factor in the aetiology is obesity and the mainline therapies are geared towards loss of excessive body weight. This observed effect may be attributed to the presence of bioactive components, specifically polyphenols such as flavonoids which provide an efficient weight loss strategy by modulating lipid metabolism and/or increasing BMR and thermogenesis [38]. They have also been reported to increase lipolysis and induce fatty acid β-oxidation through modulation of hormone sensitive lipase, acetyl-coA carboxylase, carnitine acyl transferase and peroxisome

proliferator-activated receptor gamma coactivator-1 [38]. This mechanism may be supported by the presence of unsaturated fat, low calories and high water content of *Citrullus lanatus* seeds.

The results also showed a significant decrease ($p<0.05$) in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin when compared to the control group. However, there was a significant increase in Alkaline Phosphatase (ALP) concentration. Alkaline Phosphatase is present in all the tissues of the body, but is particularly concentrated in the liver, bile duct, kidney, bone and the placenta [39] and as such is not a specific liver marker [39]. Because of the low levels of the other liver biomarkers, (AST, ALT and bilirubin) increase in ALP alone cannot characterize liver damage. Raised serum ALP level usually reflects impaired excretion and bile flow like that observed during obstruction that could affect the biliary system. Increased serum levels may reflect increased synthesis or inadequate excretion as in conditions leading to increased biliary pressure. Thus, the increase observed could not have indicated hepatobiliary obstruction; rather extra hepatic sources (e.g. bone) may be implicated [40]. However, this increase could be as a result of the phenolic glycosides of the seeds which may increase the ALP activity of human osteoblast-like cells. Overall, the decrease in liver enzymes (AST, ALT) and bilirubin suggests that *Citrullus lanatus* seed has little or no deleterious effect on the liver.

The outcome of this study showed a significant increase ($P<0.05$) in packed cell volume and mean cell volume after 10 days and also a significant increase in red blood cell count after 20 days of administration. This is an indication that *Citrullus lanatus* seed extract may have a positive effect on haemopoietic system. Packed cell volume (PCV), haemoglobin concentration, red blood cells (RBC) and mean cell volume (MCV) are important for the diagnosis of anaemia [41]. Despite the increase in these parameters (PCV, RBC and MCV), a significant decrease in haemoglobin concentration was recorded; however haemoglobin concentration was still within normal physiologic range (12.0-16.0g/dL) [42] suggesting that the animals were not anaemic and oxygen transport was not altered beyond physiologic limits. The increase in packed cell volume and red blood cell count may be attributed to the presence of bioactive constituents such as flavonoids which, as earlier stated, is abundant in *Citrullus lanatus* seeds and have been linked with improvement of haematological indices (Muhammad et al., 2014). This is also buttressed by the fact that *Citrullus lanatus* seed is rich in iron in terms of nutritional value [43] which is necessary for oxygen transport [44].

Result also showed a decrease in total and differential white blood cell count after 10days which could probably be due to sudden physiological changes following acute extract-blood interaction and not necessarily pathological. However, there was also an increase in total and differential white blood cell count, after 20 days. This increase may be attributed to increase in the production of leucocytes which may be due to the presence of flavonoids which are known to inhibit degranulation of neutrophils [45]. This inhibitory effect has been shown to be due to modulation of the receptor-directed Ca^{2+} channels in the plasma membrane [46]. Thus *Citrullus lanatus* seeds may build the immune system.

There was significant decrease in platelet count after 20 days of administration. This may be attributed to the presence of flavonoids which have anti-platelet activity as suggested by Johnson et al. [47].

5. CONCLUSION

The results of the study suggest that *Citrullus lanatus* seeds cause reduction of weight in normal Wistar rats which could be beneficial in obese conditions. Continued consumption of these seeds also had beneficial effects on haematological indices, indicating a possible alleviating effect on anaemia. Inflammatory processes implicated in the progression of metabolic disorders could also be suppressed due to the depressing effects the seeds have shown on platelet activity and leucocyte function.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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