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Effect of *Citrus aurantifolia* juice on bodyweight and haematological indices of wistar rats

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Citrus aurantifolia **(Lime) is a citrus fruit and an excellent source of vitamin C and flavonoids which have unique antioxidant properties. This study determined the effects of *C. aurantifolia* on the bodyweight and hematological indices of rats. A total of 20 rats of both sex weighing between 120 and 130 g were randomized into 4 groups of five rats each and used. Group A: Normal Control; Group B: 2 ml/kg of *C. aurantifolia* fruit juice; Group C: 4 ml/kg of *C. aurantifolia* fruit juice; Group D: 8 ml/kg of *C. aurantifolia* fruit juice. The *C. aurantifolia* fruit was freshly squeezed and the juice was administered to the test groups for a period of three months. The haematological parameters were analysed using standard methods. The results revealed that administration of *C. aurantifolia* juice caused a significant difference ($p<0.05$) in the bodyweights of the experimented groups from week 3 up to week 12 compared with the normal control group. After three months of the administration, only the platelet counts showed a significant difference ($p>0.05$) in group B compared with other groups throughout the three months of administration. Therefore, it is then concluded that bioactive substances present in lime like lycopene and vitamin C induces the proliferation of white blood cells in the blood circulation. Hence, it was proven that lime has a protective effect which may serve as an alternative treatment option in patients with leukopenia.**

Key words: Lime, three months, administration, platelet, fruit, groups.

INTRODUCTION

Food is thought about as a basic need for human. However, unhealthy food consumption degrades the quality of life instead of a balance diet. In this regard, Slavin and Lloyd (2012) stated that vegetables and fruits contain essential nutrients such as dietary fiber, carotenoids, vitamins, minerals, folate, plant sterols, and various phytochemicals are required for human daily diet. According to Wang et al. (2014); Crowe (2015) and Aune et al. (2017), these nutrients help to reduce mortality and prevent chronic diseases, including various cardiovascular

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diseases, cancers and even mental illnesses which is ascribed to the presence of different phytochemicals on those fruits and vegetables, functioning as anti-inflammatory agents, phytoestrogens, and antioxidants (Gouegni and Abubakar, 2013; Rodriguez-Casado 2016).

Enejoh et al. (2015) reported that the fruit juice of *Citrus aurantifolia* (Rutaceae), commonly known as lime, is added to sugar and palm oil or honey to relief cough and considered as tonic for libido and as antidote for poison. Aibinu et al. (2007) reported that the diluted form of the *C. aurantifolia* fruit juice is used for mouth wash to treat sore mouth and sore throat. The fruits of *C. aurantium* are sources of flavonoid-type compounds with diverse biological effects (Kang et al., 2011; Hamada et al., 2017; Liu et al., 2008) besides the essential oil and its components (Moraes et al., 2009; Barceloux, 2008). Additionally, Zhang et al. (2017) reported the isolation of flavonoid glycosides from the plant and the determination of biogenic amine and flavanone contents by Pellati et al. (2004) and Bagatela et al. (2015). Akhtar (2013) reported the use of the juice in treating irritation, diarrhoea and swelling due to mosquito bites, and is sometimes used as vermifuge when mixed with oil and also incorporated into weight management diet. Medicinal plants play important role in the management of diseases due to their rich antioxidants, phytochemicals, easy accessibility and affordability by the populace (Alaebo et al., 2020; Onochie et al., 2020; Iloanya et al., 2021).

Phytopharmaceuticals have shown an outstanding role in new drug discovery (Khan and Amin, 2016; Khan et al., 2016) in the last years and a large population globally are getting therapeutic benefits from them (Marya et al., 2018) both in the crude form as well as pure chemical entities. Kuding leaves known to be a medicinal plant has been observed in some studies to affect bodyweight and packed cell volume (Alisa et al., 2015; Ezeigwe et al., 2018). Some herbs may be beneficial in bodyweight reduction but may have adverse effect on the blood parameters. There is always needed to investigate the effect of medicinal plants on biochemical and haematological parameters before recommending them to those that need them for bodyweight reduction. One of our earlier studies investigated the effect of *C. aurantifolia* fruit juice on biochemical parameters of Wistar rats (Ezeigwe et al., 2022). There is paucity of information on the effects of *C. aurantifolia* on the blood parameters of Wistar rats, thus, the present study aimed to evaluate the effect of *C. aurantifolia* juice on bodyweight and hematological indices of rats.

MATERIALS AND METHODS

Sample collection and identification

The *C. aurantifolia* fruits were purchased from Eke Market, Awka, Awka South Local Government Area, Anambra State, Nigeria. The sample was identified by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka. The voucher number as deposited in the herbarium of Nnamdi Azikiwe University, Awka is NAUH 196[A].

Test animals

A total of 20 Wistar albino rats of both sex weighing between 120-130 g were purchased from Chris Experimental Animal Farm and Research Laboratory, Awka, Anambra State and used for the experiment. They were maintained and housed in cages under standard environmental conditions (27±3°C, 12 h light/dark cycle) in the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka. They were allowed to acclimatize with the environment for one week before use. The animals were fed vital grower's mash pellets purchased from Vital Feed Distributor at Awka, Anambra state and fed *ad libitum*. At the end of the one-week acclimatization period, the animals were weighed, grouped, and labeled.

Study design

A total of 20 rats of both sex weighing between 120 and 130 g were randomized into 4 groups of five rats each and used. The *C. aurantifolia* fruit was freshly squeezed and the juice was administered to the test groups for a period of three months.

Group A: Normal Control
Group B: 2 ml/kg bodyweight of *C. aurantifolia* fruit juice
Group C: 4 ml/kg bodyweight of *C. aurantifolia* fruit juice
Group D: 8 ml/kg bodyweight of *C. aurantifolia* fruit juice

Determination of bodyweight

The weight of the experimental subjects was checked using an electronic weighing scale. The bodyweights of the rats were monitored before, during, and after the experiment to know whether the continuous administration of lime juice caused a noticeable increase or decrease in bodyweight. Percentage bodyweight was also calculated using the formular below:

$$\text{Percentage weight} = \frac{Weekly\ \ weig\,ht - Initial\ \ weig\,ht}{Initial\ \ weig\,ht} \times \frac{100}{1}$$

Haematological analysis

Haematological parameters were determined using automated haematology analyzer (Mindray-BC-5300). The haematological parameters that were analysed include haemoglobin (HGB), packed cell volume (PCV), red blood cells (RBC), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MON), eosinophils (EOS), basophils (BAS).

Statistical analysis of results

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for windows version 23 (SPSS Inc., Chicago, Illinois, USA). All the data collected were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA Tests to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

Table 1. Weekly Bodyweight of rats administered different doses of *C. aurantifolia* fruit juice.

Groups	Weight (g) Week 0	Weight (g) Week 1	Weight (g) Week 2	Weight (g) Week 3	Weight (g) Week 4	Weight (g) Week 5	Weight (g) Week 6	Weight (g) Week 7	Weight (g) Week 8	Weight (g) Week 9	Weight (g) Week 10	Weight (g) Week 11	Weight (g) Week 12
Normal control	123.72±2.59	130.11±2.33	139.06±1.86[a]	146.64±1.32[a]	154.20±1.65[a]	162.71±1.18[a]	170.63±1.06[a]	181.57±1.30[a]	190.61±2.13[a]	197.74±2.57[a]	205.12±1.32[a]	211.41±2.46[a]	219.35±2.68[a]
2 ml/kg bw. of *C. aurantifolia* fruit juice	125.41±1.23	131.73±3.99	134.31±2.02	138.53±1.93[a]	143.20±0.91[a]	149.82±2.21[a]	151.34±2.07[a]	146.12±2.12[a]	140.45±2.87[a]	137.02±1.14[a]	136.37±1.50[a]	132.14±1.31	126.82±2.64
4 ml/kg bw. of *C. aurantifolia* fruit juice	121.91±1.07	126.42±1.53	129.76±1.61	133.23±2.03	136.52±1.35[a]	138.51±2.75[a]	137.23±1.57[a]	134.67±1.34[a]	130.24±1.37	128.52±2.29	125.16±1.34	124.78±2.18	120.30±1.39
8 ml/kgbw. of *C. aurantifolia* fruit juice	123.23±2.62	130.65±1.11	134.32±2.72	139.21±2.16[a]	140.98±1.83[a]	140.25±0.65[a]	138.72±1.52[a]	136.32±1.26[a]	135.90±1.14[a]	130.64±2.06	127.02±1.46	126.23±1.77	121.86±1.43

[a]Significant increase with respect to week 0; [b]Significant decrease with respect to week 0.
Source: Authors

Table 2. Percentage increase/decrease in bodyweight of rats administered different doses of *C. aurantifolia* fruit juice.

Groups	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Normal Control	-	5.17	12.37	18.51	24.66	31.53	37.91	46.89	54.08	59.82	65.80	70.90	77.28
2 ml/kg bw. of *C. aurantifolia* fruit juice	-	5.02	7.10	10.45	14.19	19.46	20.65	16.51#	11.96#	9.25#	8.69#	5.34#	1.12#
4 ml/kg bw. of *C. aurantifolia* fruit juice	-	3.69	6.40	9.27	11.98	13.62	12.55#	10.42#	6.81#	5.41#	2.63#	2.30#	1.31#
8 ml/kg bw. of *C. aurantifolia* fruit juice	-	6.01	9.01	12.99	14.37	13.80#	12.58#	10.63#	10.31#	6.01#	3.08#	2.44#	1.14#

*increase; #decrease.
Source: Authors

RESULTS

Effect on bodyweight

The body weight of rats administered different doses of *C. aurantifolia* fruit was recorded as shown in Table 1. Group A (Normal control) showed an increase in bodyweight from the baseline, which steadily increased up to the 12th week. Group B showed an increased-decreased pattern in bodyweight. It increased from the baseline, which increased steadily up to week 6, followed by a sharp decrease in week 7, which continued till the 12th week. Group C and D followed the same increased-decreased pattern, with a decrease in bodyweight manifesting from week 6 (Table 1). The percentage increase/ decrease in bodyweight of rats was calculated and shown in Table 2. It showed a significant ($p<0.05$) increase in the bodyweight of the normal control (Group A) compared with the test groups (Groups B, C and D).

Haematological analysis

Table 3 showed the Hemoglobin (HGB) concentration of rats administered with *C. aurantifolia*. HGB of the subjects from Group A (Normal control) and Group B decreased from their baselines after 1st month, followed by a further decrease in the 2nd month but increased in the 3rd month. Group C showed a decrease from its baseline after the 1st month, peaked in the 2nd month and even higher in the3rd month. Group D

Table 3. Haemoglobin concentration of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	HGB (g/dl)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	14.93±0.07	13.81±0.03	13.30±0.09	14.75±0.01
2 ml/kg bw. of *C. aurantifolia* fruit juice	14.63±0.03	14.15±0.02	13.03±0.05	14.12±0.06
4 ml/kg bw. of *C. aurantifolia* fruit juice	13.75±0.10	13.62±0.03	13.69±0.03	14.32±0.03
8 ml/kg bw. of *C. aurantifolia* fruit juice	14.02±0.15	13.23±0.02	13.96±0.02	13.85±0.03

Source: Authors

Table 4. Haematocrit of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	HCT (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal control	47.83±2.20	46.12±2.51	43.54±2.80	47.27±1.32
2 ml/kg bw. of *C. aurantifolia* fruit juice	45.22±1.93	46.04±1.14	42.81±1.43	45.04±2.12
4 ml/kg bw. of *C. aurantifolia* fruit juice	42.35±0.58	43.67±1.83	43.93±3.11	45.01±0.52
8 ml/kg bw. of *C. aurantifolia* fruit juice	46.96±1.97	43.13±0.93	44.02±1.07	43.27±0.17

Source: Authors

Table 5. Mean Corpuscular Volume of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	MCV (fl)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	60.76±3.08	60.35±1.82	60.57±3.36	59.54±1.81
2 ml/kg bw. of *C. aurantifolia* fruit juice	60.86±1.60	60.32±1.30	59.64±1.83	60.20±3.03
4 ml/kg bw. of *C. aurantifolia*fruit juice	59.23±0.98	60.21±1.23	58.53±2.42	58.63±0.71
8 ml/kg bw. of *C. aurantifolia*fruit juice	58.87±2.76	59.49±2.94	58.41±0.96	56.12±1.63

Source: Authors

Table 6. Mean Corpuscular Haemoglobin of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	MCH (pg)			
	Initial	1st Month	2nd Month	3rd Month
Normal control	19.01±0.02	18.73±0.18	18.76±0.03	18.51±0.01
2 ml/kg bw. of *C. aurantifolia* fruit juice	18.57±0.09	18.36±0.03	18.51±0.12	18.74±0.03
4 ml/kg bw. of *C. aurantifolia*fruit juice	18.91±0.03	18.72±0.01	18.63±0.03	18.79±0.01
8 ml/kg bw. of *C. aurantifolia* fruit juice	18.75±0.11	18.22±0.03	18.95±0.02	18.63±0.25

Source: Authors

followed the same pattern as in Group C with a decrease in the 3rd month. Table 4 showed the Hematocrit concentration of rats administered with *C. aurantifolia*. HCT of the subject from Group A (Normal control) decreased from its baseline after 1st month, followed by a further decrease in the 2nd month but increased in the 3rd month. HCT of the subject from Group B increased from its baseline after 1st month, followed by a sudden decrease in the 2nd month but shot up in the 3rd month. Group C increased from its baseline after 1st month, which steadily increased in the 2nd month, up till the 3rd month. Group D showed a decreased and increased pattern after 1st month, 2nd month and 3rd month, respectively.

MCV results were shown in Table 5. Although there was a decrease in all the groups in the 3rd month of administration from their baselines, no significant differences ($p>0.05$) were observed throughout the three months of administration with Lime Juice. MCH results were shown in Table 6. There were no significant

Table 7. Mean Corpuscular Haemoglobin Concentration of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	MCHC (g/dl)			
	Initial	1st Month	2nd Month	3rd Month
Normal control	31.22±0.01	32.64±0.32	31.83±0.03	32.05±0.07
2 ml/kg bw. of *C. aurantifolia*fruit juice	31.62±0.03	33.20±0.03	31.73±0.04	31.01±0.03
4 ml/kg bw. of *C. aurantifolia*fruit juice	33.14±0.15	30.82±0.07	32.57±0.03	31.97±0.02
8 ml/kg bw. of *C. aurantifolia* fruit juice	32.71±0.02	31.66±0.01	33.25±0.05	33.19±0.02

Source: Authors

Table 8. Red Blood Cell count of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	RBC (× $10^{12/L}$)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	7.84±0.28	7.53±0.21	6.91±0.04	7.62±0.04
2 ml/kg bw. of *C. aurantifolia* fruit juice	7.61±0.06	7.50±0.06	7.78±0.02	7.53±0.01
4 ml/kg bw. of *C. aurantifolia* fruit juice	7.41±0.07	7.26±0.03	7.63±0.03	7.68±0.61
8 ml/kg bw. of *C. aurantifolia* fruit juice	7.23±0.13	7.15±0.09	7.32±0.03	7.49±0.03

Source: Authors

Table 9. Platelet count of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	PLT (× $10^{9/L}$)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	869.56±3.18	756.57±3.14	796.20±2.81	823.13±2.15
2mL/kg bw. of *C. aurantifolia*fruit juice	696.30±5.62	730.12±3.12[c]	826.35±4.92[e]	933.75±3.26[g]
4mL/kg bw. of *C. aurantifolia*fruit juice	811.32±2.87	813.63±5.49	792.57±2.28	627.16±5.10[h]
8mL/kg bw. of *C. aurantifolia* fruit juice	720.18±5.30	652.92±3.72[d]	691.71±2.23	585.41±3.22[h]

[c]Significant increase with respect to 1st Month; [d]Significant decrease with respect to 1st Month; [e]Significant increase with respect to 2nd Month; [f]Significant decrease with respect to 2nd Month; [g]Significant increase with respect to 3rd Month; [h]Significant decrease with respect to 3rd Month.
Source: Authors

differences (p>0.05) observed throughout the three months of administration with lime Juice as the same level was maintained in all the groups. The results in Table 7 showed the MCHC of rats administered with different doses of *C. aurantifolia* fruit juice. There were no significant differences (p>0.05) observed throughout the three months of administration with lime Juice.

Red blood cell count (RBC) of rats administered with *C. aurantifolia* is shown in Table 8. RBC of the subject from Group A (Normal control) decreased from its baseline after 1st month, followed by a further decrease in the 2nd month but peaked in the 3rd month. Group B showed a decreased and increased pattern after 1st month, 2nd month and 3rd month, respectively. Group C and D decreased from their baselines after 1st month, which steadily increased in the 2nd month, up till the 3rd month.

Platelet count (PLT) of rats administered with *C. aurantifolia* is shown in Table 9. PLT of the subject from Group A (Normal control) decreased from its baseline after 1st month, followed by a sharp increase in the 2nd month up till the 3rd month. Group B increased from its baseline after 1st month, which steadily increased in the 2nd month, up till the 3rd month. Group C increased from its baseline after 1st month, followed by a sharp decline in the 2nd month, which further decreased in the 3rd month. Group D showed a decreased and increased pattern after 1st month, 2nd month and 3rd month, respectively. All platelet counts in both Normal control and experimented groups are within the normal range (NV=500-1300 × 10^9/L) according to Barrientos et al. (2020).

White blood cell count (WBC) of rats administered with *C. aurantifolia* is shown in Table 10. WBC of the subject from Group A (Normal control) decreased from its baseline after 1st month, followed by a further decrease in the 2nd month and peaked in the 3rd month. Group B displayed an increased and decreased pattern after 1st month, 2nd month and 3rd month, respectively. Group C decreased from its baseline after 1st month, followed by a

Table 10. White Blood Cell count of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	WBC (× $10^{9/L}$)			
	Initial	1st Month	2nd Month	3rd Month
Normal control	10.91±0.11	10.54±0.30	8.62±0.51	10.35±2.12
2 ml/kg bw. of *C. aurantifolia*fruit juice	6.82±0.66	8.93±1.21	7.21±0.29	11.29±3.04g
4 ml/kg bw. of *C. aurantifolia*fruit juice	10.15±0.83	8.38±1.82	10.54±1.32	11.10±1.71
8 ml/kg bw. of *C. aurantifolia* fruit juice	8.65±0.91	9.13±1.26	10.62±0.67	13.29±1.38g

[c]Significant increase with respect to 1st Month; [d]Significant decrease with respect to 1st Month; [e]Significant increase with respect to 2nd Month; [f]Significant decrease with respect to 2nd Month; [g]Significant increase with respect to 3rd Month; [h]Significant decrease with respect to 3rd Month.
Source: Authors

Table 11. Neutrophils of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	NEU (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	4.86±1.03	3.89±0.31	4.12±0.32	3.70±0.29
2 ml/kg bw. of *C. aurantifolia* fruit juice	4.63±0.37	4.33±1.24	4.65±1.32	3.14±0.25
4 ml/kg bw. of *C. aurantifolia* fruit juice	4.37±0.31	4.10±0.12	3.23±1.00	3.32±0.15
8 ml/kg bw. of *C. aurantifolia*fruit juice	4.90±0.35	4.73±0.01	3.97±0.26	4.83±0.11

Source: Authors

Table 12. Lymphocytes of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	LYM (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	94.83±2.31	95.31±1.62	94.20±2.90	97.16±1.32
2 ml/kg bw. of *C. aurantifolia*fruit juice	92.67±2.57	96.53±1.14	93.04±3.21	96.72±1.52
4 ml/kg bw. of *C. aurantifolia*fruit juice	94.51±1.98	93.75±1.67	95.16±1.64	94.37±1.91
8 ml/kg bw. of *C. aurantifolia* fruit juice	96.14±2.02	95.83±1.39	96.39±1.54	94.13±2.46

Source: Authors

sudden increase in the 2nd month, up till the 3rd month. Group D increased steadily from its baseline after the 1st month and continued till the 3rd month. All white blood cell counts in both Normal control and experimented groups are within the normal range (NV=6-18 × 10^9/L) according to Barrientos et al. (2020).

Neutrophils of rats administered with *C. aurantifolia* were shown in Table 11. Group A (Normal control) and Group B displayed a decreased and increased pattern after the 1st month, 2nd month and 3rd month. Groups C and D increased from their baselines in the 1st month, followed by a further decrease in the 2nd month and peaked in the 3rd month.

Lymphocytes of rats administered with *C. aurantifolia* were shown in Table 12. Group A (Normal control) and Group B displayed an increased and decreased pattern after the 1st month, 2nd month and 3rd month. Group C and Group D displayed a decreased and increased pattern after the 1st month, 2nd month and 3rd month.

Monocytes of rats administered with *C. aurantifolia* were shown in Table 13. Group A (Normal control) revealed an absence of the cell throughout the testing time. Group B revealed an absence of the cell in its baseline, 1st, and 3rd month except in the 2nd month. Group C revealed a decrease from its baseline after 1st month, which peaked in the 2nd month, with an absence of the cell in the 3rd month. Group D revealed the presence of the cell after the 3rd which increased significantly ($p<0.05$) from its baseline.

Eosinophils of rats administered with *C. aurantifolia* were shown in Table 14. A decreased pattern of the cell and an increased pattern of the cell throughout the experiment were observed in Group A (Normal control) and Group D, respectively. Group B decreased from its baseline after 1st month, followed by a further decrease in the 2nd month, and peaked in the 3rd month. Group C showed a decreased and increased pattern after the 1st month, 2nd month and 3rd month, respectively. There were

Table 13. Monocytes of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	MON (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2 ml/kg bw. of *C. aurantifolia* fruit juice	0.00±0.00	0.00±0.00	0.13±0.00[e]	0.00±0.00
4 ml/kg bw. of *C. aurantifolia* fruit juice	0.20±0.00	0.12±0.00[d]	0.25±0.00	0.00±0.00
8 ml/kg bw. of *C. aurantifolia* fruit juice	0.13±0.00	0.00±0.00	0.00±0.00	0.40±0.00[g]

[c]Significant increase with respect to 1st Month; [d]Significant decrease with respect to 1st Month; [e]Significant increase with respect to 2nd Month; [f]Significant decrease with respect to 2nd Month; [g]Significant increase with respect to 3rd Month; [h]Significant decrease with respect to 3rd Mont.
Source: Authors

Table 14. Eosinophils of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	EOS (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	0.46±0.00	0.40±0.00	0.35±0.00	0.21±0.00[h]
2 ml/kg bw. of *C. aurantifolia*fruit juice	0.33±0.00	0.22±0.00[d]	0.16±0.00[f]	0.25±0.00[h]
4 ml/kg bw. of *C. aurantifolia* fruit juice	0.51±0.00	0.36±0.00[d]	0.43±0.00[f]	0.42±0.00[h]
8 ml/kg bw. of *C. aurantifolia* fruit juice	0.43±0.00	0.44±0.00	0.65±0.00[e]	0.99±0.01[g]

[c]Significant increase with respect to 1st Month; [d]Significant decrease with respect to 1st Month; [e]Significant increase with respect to 2nd Month; [f]Significant decrease with respect to 2nd Month; [g]Significant increase with respect to 3rd Month; [h]Significant decrease with respect to 3rd Month.
Source: Authors

Table 15. Basophyls of rats administered different doses of *C. aurantifolia* fruit juice.

Parameter	BAS (%)			
	Initial	1st Month	2nd Month	3rd Month
Normal Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2 ml/kg bw. of *C. aurantifolia*fruit juice	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4 ml/kg bw. of *C. aurantifolia*fruit juice	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
8 ml/kg bw. of *C. aurantifolia* fruit juice	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Source: Authors

no observed changes in the basophyls levels of the different groups of rats starting from the normal control to the experimental groups (Table 15). Table 15 revealed the absence of basophyls in rats adiministered with different doses of *C. aurantifolia* fruit juice.

DISCUSSION

A reduction in bodyweight and food consumption was observed following the intake of *C. aurantifolia* fruit juice in rats after 12 weeks (Table 1). However, this decrease was only observed in the group of rats administered 4 and 8 ml/kg bodyweight of *C. aurantifolia* after 12 weeks of administration. This entails that lime juice can be a good regimen in the management of bodyweight at certain doses. However, some medicinal plants such as *Justicia secunda* Vahl leaves (Onochie et al., 2020) do not have the capacity to cause observable changes in the bodyweight of experimental animals. It is possible that phytoestrogens of *C. aurantifolia* inhibit serotonin re-uptake and thereby increase the levels of serotonin in synaptic clefts as in the case of *C. cyminum L*. which was reported by (Amin and Nagy, 2009), which in turn would result in enhanced satiety. Additionally, bodyweight may be reduced by limonene through increased lipolysis by a histaminergic response and reduced appetite (Shen et al., 2005).

The HGB concentration results obtained and represented in Table 3 were all within the normal range (NV=11-19.2 g/dL) according to Barrientos et al. (2020), and no significant difference ($p > 0.005$) was recorded

throughout the three months of administration with lime juice. It suggests that administration with lime juice at different dosages used does not have any negative impact on HGB concentration and could be effective in the treatment of anaemia. Blood indices (MCV, MCH, and MCHC) results as shown in Tables 5, 6 and 7 respectively showed no significant differences ($p>0.05$) throughout the three months of administration with lime Juice. There was no significant difference ($p>0.05$) observed in RBC counts of both normal control and groups administered with lime juice (Table 8), and the values were within the normal range (NV= 6.76-9.75 × 10^{12}/L) according to Barrientos et al. (2020). The steady values of RBC could be attributed to the rich milieu of antioxidants that protects the RBC from any oxidative damage.

Platelets are small, colorless cell fragments in our blood that form clots and stop or prevent bleeding. The result in table 9 revealed a significant increase ($p<0.05$) in group B relative to other administered groups and Normal control (Group A) after 3rd month of lime juice administration. The lowest value was found in group D after 3rd month of lime juice administration. The results although either showing a decreasing or increasing patterns in the different groups administered with lime juice were all with the normal range (NV=500-1300 × 10^{9}/L) according to Barrientos et al. (2020), hence neither thrombocytosis nor thrombocytopenia can be implied.

Eyong et al. (2004) reported that rapid increase in WBC counts following a foreign attack on the system by pathogens gives rise to a boost in the body's defense mechanisms as the normal physiological response. After 3rd month of administration of lime juice, it was observed that the administered groups showed significant ($p<0.05$) increases relative to their baselines (Table 10). This could imply that lime juice could serve as an immune booster. The effects of the prolonged administration of lime fruit juice on the white blood cells differentials were also investigated. Following the results obtained at monthly intervals, there was no significant difference ($p<0.05$) in the neutrophil and lymphocyte levels of the administered groups. However, monocytes and eosinophils revealed an inconsistent increase and decrease at different doses analysed at monthly intervals (Tables 13 and 14). The white blood cell and its differentials are vital for the protection of the body against foreign invaders and help to stimulate cytokine erythropoietin which subsequently stimulates blood cell synthesis (Oladejo and Osukoya, 2021).

Conclusion

The domestic and ethnomedical use of *C. aurantifolia* cannot be overemphasized. The observations from this study revealed that fruit juice of *C. aurantifolia* not only could serve as immune booster but can also be used in bodyweight management. There should be a move from pre-clinical screening of the plant's fruit juice to the isolation of active compounds and the actual development of useful drugs from the plant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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