

# FUDMA Journal of Sciences (FJS) ISSN online: 2616-1370 ISSN print: 2645 - 2944 Vol. 9 No. 10 2025, pp 393 - 397

Vol. 9 No. 10 2025, pp 393 - 397

DOI: https://doi.org/10.33003/fjs-2025-0912-4371



# DIETARY SUPPLEMENTATION WITH CITRULLUS LANATUS WHOLE FRUIT AND PULP POSSESSES ANTIOXIDANT EFFECTS IN OXIDATIVE STRESSED RATS

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#### **ABSTRACT**

The pulp of Citrullus lanatus is mostly consumed while the seeds and rind are discarded. This study evaluated the effect of a diet supplemented with C. lanatus whole fruit and pulp against acetaminophen induced oxidative stress in rats. Whole fruits and pulp were cut into small sizes, air-dried and grounded. Twenty rats were divided into four groups of five each. Group 1(normal control) and 2 (negative control) consumed normal rat chow while groups 3 and 4 consumed normal rat chow supplemented with 5% C. lanatus whole fruit and pulp respectively for 21 days. Haematological parameters were monitored weekly. On the 21st day, a single dose of acetaminophen (2g/kg) was administered to groups 2, 3, and 4. All rats were humanely sacrificed 12 hours later. Haematological parameters and antioxidant enzymes were measured in the blood and liver respectively Results showed that both supplementation with 5% whole fruit and pulp had no significant (p>0.05) effect on haematological parameters. However, acetaminophen overdose caused a significant (p<0.05) decrease in the haematological parameters of rats in group 2, and also decreased the levels of the biomarkers (SOD, Catalase, GSH) for oxidative stress. Groups supplemented with 5% whole fruit and pulp showed a significantly smaller reduction (p<0.05) in haematological parameters compared to the negative control, with their final values being significantly higher. Furthermore, the levels of SOD, catalase and GSH were higher (p<0.05) in these supplemented groups. Therefore, dietary supplementation with Citrullus lanatus whole fruit and pulp possesses antioxidant effect with the 5% pulp supplementation demonstrating a superior effect on hepatic antioxidant biomarkers.

Keywords: Acetaminophen, Citrullus lanatus, Dietary supplementation, Oxidative stress, Haematology

# INTRODUCTION

Fruits and vegetables are rich sources of phytochemicals. These phytochemicals illicit various beneficial properties to health; they possess antioxidant properties, the ability to inhibit gene expression, the induction of enzymes, receptor inhibition activities and haematinic activities. (Karasawa and Mohan, 2018; Santos *et al.*, 2019). Therefore, fruits and vegetables are widely consumed due to their benefits to health.

Citrullus lanatus commonly called watermelon is a member of the Cucurbitaceae family and it is widely cultivated in the tropics (Deshmukh and Tambe, 2015). Watermelon is rich in phytochemicals such as carotenoids, vitamins, alkaloids and phenolic compounds in its various parts in varying concentrations (Alemika et al., 2018, Siddiqui et al., 2018, Jibril et al., 2019).

Paracetamol (acetaminophen), a commonly used analgesic and antipyretic agent, can cause oxidative stress and its related complications when used excessively (Larsen *et al.*, 2014, Ommati *et al.*, 2017). Oxidative stress is caused by excessive generation of reactive oxygen species and an imbalance in the antioxidant levels (Butt *et al.*, 2009); thus, acetaminophen overdose causes depletion in the levels of GSH in the hepatic cells, thereby causing oxidative stress (Lancaster *et al.*, 2015). In addition, uncontrolled oxidative stress could interfere with a lot of cellular processes and alter the structure of major biomolecules, including the blood cells (Prakasam *et al.*, 2001). Interestingly, plants and their derivatives have the potential to ameliorate oxidative stress and complications associated with it (Tian *et al.*, 2023).

Reports have shown the beneficial effects of various parts of *C. lanatus*. The roots and leaves are reported to have analgesic and anti-inflammatory effects, while the fruit and seeds are reported to have antioxidant, anti-ulcer and antihypertensive abilities (Mladenović *et al.*, 2014; Men *et al.*, 2020; Sifuna, 2022). However, only the pulp of watermelon is often consumed while the rinds and seeds are discarded (Egbuonu *et al.*, 2016). Therefore, this study was aimed at investigating the haematological and antioxidant effect of *C. lanatus* whole fruit supplementation in rats with acetaminophen-induced oxidative stress.

# MATERIALS AND METHODS

# **Collection of Fruit Sample**

Citrullus lanatus whole fruit was obtained from Samaru, Sabon Gari local government area of Kaduna State, Nigeria. The plant was authenticated at the herbarium unit of the Department of Botany, Ahmadu Bello University (A.B.U), Zaria (Voucher Number: 2234).

## **Experimental Animals**

Twenty adult albino rats of either sex weighing 100g - 150g were obtained from the animal house, Faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria. The rats were kept in cages with free access to food and water *ad libitum* at room temperature and under a natural light-dark cycle. The rats were allowed to acclimatize for two weeks before the experiment. Ethical approval for the study was obtained (Ethical approval number: ABUCAUC/2025/016), and animal handling and

protocol were carried out following the Ethical Review Committee for animal use and care of Ahmadu Bello University.

### **Preparation of the Fruit Sample**

The whole fruit and pulp of *Citrullus lanatus* were separately washed, then cut into smaller sizes, dried under room temperature and then ground into powder using a dry blender. The grounded samples were used for the supplemental feeding.

# **Preparation of the Supplemented Feeds**

Fifty grams (50g) of each of the fruit samples were mixed with 950g of the normal rat chow, which was thoroughly mixed to make 1kg each of the supplemented samples.

# Animal Grouping and Acetaminophen Administration

The rats were divided into four groups of five rats each.

Group 1: Normal Control; fed on normal rat chow

Group 2: Negative control; fed on normal rat chow

Group 3: rats fed on normal rat chow supplemented with 5% *C. lanatus* whole fruit.

Group 4: rats fed on normal rat chow supplemented with 5% *C. lanatus* pulp.

Supplementary feeding was done for 21 days, with weekly determination of haematological parameters. On the 21<sup>st</sup> day, a single dose of acetaminophen (2g/kg) was given by oral gavage 12 hours before sacrifice to groups 2, 3 and 4. Rats were humanely sacrificed, and blood samples were collected, and livers were harvested.

#### **Blood Collection**

Blood was collected weekly (days 7, 14, and 21) for haematological analysis. The packed cell volume (PCV), haemoglobin (Hb) concentration, blood platelets, red blood cell (RBC) count and white blood cell (WBC) count were determined using the Automated Haematological Analyzer.

# Harvest and Homogenization of Liver Tissue

At the end of all treatments, the rats were anesthetized and sacrificed humanely, blood and liver tissues were collected. The livers were quickly excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination and kept on ice. 0.1M sodium phosphate buffer (pH 7.4) was used for homogenization. 1g of the organ was homogenized in 10ml of the buffer using a pestle and mortar. The homogenate was then centrifuged at 3000rpm for 10 minutes and the supernatant was collected using a Pasteur pipette.

# **Determination of Oxidative Stress Markers**

Catalase activity was measured by the method of Aebi (1974), Superoxide Dismutase (SOD) was measured according to (Fridovich, 1989), while the assay for glutathione Concentration was done following Ellman, 1959 protocol.

# RESULTS AND DISCUSSION Results

Table 1 shows a significant (p<0.05) increase in the level of SOD, GSH and catalase in the groups supplemented with C. lanatus when compared to the negative control group. However, the increase was remarkably higher in the groups supplemented with 5% pulp of C. lanatus.

Table 1: Effect of Citrullus Lanatus Whole Fruit and Pulp Dietary Supplementation On Hepatic Antioxidant Biomarkers of Rats

Group	SOD (U/mg protein)	Catalase (U/mg protein)	GSH (μg/ml)
Normal control	$88.60 \pm 8.46^{c}$	$18.33 \pm 6.28^{a}$	$75.24 \pm 1.80^{b}$
Negative control	$51.75 \pm 4.02^{a}$	$17.02 \pm 4.33^{a}$	$67.49 \pm 3.48^{a}$
5% Whole Fruit	$60.00 \pm 7.76^{ab}$	$19.18 \pm 43.44^{a}$	$80.38 \pm 3.79^{c}$
5% Pulp	$71.05 \pm 16.43^{b}$	$27.87 \pm 9.46^{a}$	$79.82 \pm 2.58^{bc}$

n=5. Data are presented as the mean  $\pm$  SD (n=5). <sup>a-b</sup>values with different superscript alphabets along a column are significantly (p< 0.05) different from each other. SOD: Superoxide dismutase

The effect of *Citrullus lanatus* whole fruit and pulp supplementation on Haemoglobin concentration of the rats is presented in Table 2. The result shows that there was no significant (p<0.05) difference in the haemoglobin concentration of rats in all the groups on day 0, 7 and 14. However, on day 21, there was a significant (p<0.05) decrease in haemoglobin concentration of rats in the negative control, and

the supplemented groups when compared with the normal control; however, groups supplemented with 5% whole fruit, and 5% pulp had significantly (p<0.05) higher concentration when compared to the negative control. Also, the haemoglobin concentration was significantly (p<0.05) higher in rats in the 5% Whole fruit group when compared to the rats in the 5% pulp group.

Table 2: Effect of Citrullus Lanatus Whole Fruit and Pulp Dietary Supplementation On the Haemoglobin Concentration of Oxidatively Stressed Rats

GROUPS	DAYS			
	0	7	14	21
Normal C	$13.20\pm0.18^{a}$	$14.25 \pm 0.13^a$	$14.78 \pm 0.09^{a}$	$14.65 \pm 0.13^{d}$
Negative C	$13.35 \pm 0.13^{a}$	$14.25 \pm 0.12^{a}$	$14.83 \pm 0.08^a$	$12.25 \pm 0.12^{a}$
5% W	$13.25 \pm 0.13^{a}$	$14.30 \pm 0.08^{a}$	$14.82 \pm 0.10a$	13.45 ±0.21 <sup>b</sup>
5% P	$13.35 \pm 0.12^a$	$14.35 \pm 0.13^{a}$	$14.81 \!\pm 0.08^a$	$12.78 \pm 0.10^{a}$

n=5. Values are represented as mean  $\pm$  standard deviation. Means with different superscript down the column are statistically significant (p<0.05). Normal C: normal control, Negative C: negative control, 5%W: 5% *Citrullus lanatus* whole fruit, 5% P: 5% *Citrullus lanatus* pulp

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The effect of *Citrullus lanatus* whole fruit and pulp supplementation on the red blood cell concentration of the rats is presented in Table 3. There was no significant (p<0.05) difference in the red blood cell count of rats in all the groups on day 0, 7 and 14. However, on day 21 there was a significant (p<0.05) decrease in RBC count of rats in the negative control,

and the supplemented groups, when compared with the normal control, however, groups supplemented with 5% whole fruit, and 5% pulp had significantly (p<0.05) higher counts when compared to the negative control. Also, there was no significant (p<0.05) difference in red blood cell count of rats supplemented with 5% whole fruit and 5% pulp.

Table 3: Effect of Citrullus Lanatus Whole Fruit and Pulp Dietary Supplementation On the Red Blood Cell Count of Oxidatively Stressed Rats

GROUPS	DAYS			
	0	7	14	21
Normal C	$6.85\pm0.13^{a}$	$5.75\pm0.12^{a}$	6.18± 0.09a	6.25 ±0.12 <sup>b</sup>
Negative C	$5.88 \pm 0.10^{a}$	$5.74 \pm 0.13^{a}$	$6.17\pm0.10^{a}$	$5.23\pm0.15^{a}$
5% W	$5.93\pm0.15^{a}$	$5.73 \pm 0.14^{a}$	$6.30 \pm 0.08^{a}$	$5.65 \pm 0.13^{a}$
5% P	$5.83\pm0.10^{a}$	$5.83\pm0.10^{a}$	$6.28 \pm 0.13^{a}$	$5.57 \pm 0.10^{a}$

n=5. Values are represented as mean  $\pm$  standard deviation. Means with different superscript down the column are statistically significant (p<0.05). Normal C: normal control, Negative C: negative control, 5%W: 5% *Citrullus lanatus* whole fruit, 5% P: 5% *Citrullus lanatus* pulp

The effect of *Citrullus lanatus* whole fruit and pulp supplementation on the white blood cell concentration of the rats is presented in Table 4. The result shows no significant (p<0.05) difference in the white blood cell count of rats in all the groups on day 0, 7 and 14. However, on day 21 there was a significant

(p<0.05) increase in the WBC count of rats in the negative control, and the supplemented groups when compared with the normal control, however, groups supplemented with 5% whole fruit, had significantly (p<0.05) lower counts when compared to the negative control.

Table 4: Effect of Citrullus Lanatus Whole Fruit and Pulp Dietary Supplementation On the White Blood Cell Count of Oxidatively Stressed Rats

GROUPS	DAYS			
	0	7	14	21
Normal C	$4.18\pm0.13^{a}$	$4.25\pm0.13^{a}$	$4.35\pm0.12^{a}$	$4.23\pm0.09^{a}$
Negative C	$4.20\pm0.16^{a}$	$4.33 \pm 0.17^{a}$	$4.25 \pm 0.13^{a}$	$4.88 \pm 0.10^{c}$
5% W	$4.05\pm0.13^{a}$	$4.32 \pm 0.18^{a}$	$4.32\pm0.17^{a}$	$4.58 \pm 0.10^{b}$
5% P	$4.23 {\pm}~0.17^a$	$4.31 \pm 0.19^{a}$	$4.26 \pm 0.12^a$	$4.75 \pm 0.12^{c}$

n=5. Values are represented as mean  $\pm$  standard deviation. Means with different superscript down the column are statistically significant (p<0.05). Normal C: normal control, Negative C: negative control, 5%W: 5% *Citrullus lanatus* whole fruit, 5% P: 5% *Citrullus lanatus* pulp

The effect of *Citrullus lanatus* whole fruit and pulp on packed cell volume of albino rats is presented in Table 5. While there was no significant (p<0.05)) difference in the packed cell volume of rats in all the groups on day 0, 7 and 14, there was a significant (p<0.05) decrease in the packed cell volume of rats in the negative control, and the supplemented groups when

compared with the normal control, however, groups supplemented with 5% whole fruit, and 5% pulp had significantly (p<0.05) higher values when compared to the negative control. Results showed that packed cell volume was significantly (p<0.05) higher in rats in the 5% whole fruit when compared to the rats in the 5% pulp group.

Table 5: Effect of Citrullus Lanatus Whole Fruit and Pulp Dietary Supplementation On the Packed Cell Volume of Oxidatively Stressed Rats

GROUPS	DAYS			
	0	7	14	21
Normal C	$40.25\pm0.96^{a}$	44.75± 0.96a	$46.25\pm0.10^{a}$	47.25± 1.50 <sup>d</sup>
Negative C	$41.00\pm0.81^{a}$	$45.00\pm0.82^{a}$	$46.25 \pm 0.90^a$	$37.50\pm1.29^{a}$
5% W	$40.75\pm1.71^{a}$	$45.00\pm1.41^{a}$	$47.50\pm1.29^{a}$	42.00±1.83°
5% P	$41.25\pm 0.96^{a}$	$44.50\pm1.05^{a}$	$46.25 \pm 0.96^a$	39.75±0.96 <sup>b</sup>

n=5. Values are represented as mean  $\pm$  standard deviation. Means with different superscript down the column are statistically significant (p<0.05). Normal C: normal control, Negative C: negative control, 5%W: 5% *Citrullus lanatus* whole fruit, 5% P: 5% *Citrullus lanatus* pulp

The effect of *Citrullus lanatus* whole fruit and pulp on the platelets count of oxidatively stressed rats is presented in Table 6. It shows that there was no significant (p<0.05) difference in

the platelets counts of rats in all the groups on day 0, 7, 14. Furthermore, acetaminophen overdose on day 21 had no significant (p<0.05) effect on the platelets count.

Table 6: Effect of Citrullus Lanatus Whole Fruit and Pulp Dietary Supplementation On the Platelets Count of Oxidatively Stressed Rats

GROUPS	DAYS			
	0	7	14	21
Normal C	7.28 ±0.17 <sup>a</sup>	$7.15\pm0.13^{a}$	$7.18\pm0.09^{a}$	7.13 ±0.10 <sup>a</sup>
Negative C	$7.30\pm 0.14^{a}$	$7.25\pm0.12^{a}$	$7.23 \pm 0.10^{a}$	$7.23 \pm 0.09^{a}$
5% W	$7.25 \pm 0.13^{a}$	$7.26 \pm 0.11^{a}$	$7.20 \pm 0.08^{a}$	$7.20 \pm 0.14^{a}$
5% P	$7.18 \pm 0.17^{a}$	$7.24\pm0.13^{a}$	$7.30 \pm 0.10^{a}$	$7.15 \pm 0.13^{a}$

n=5. Values are represented as mean  $\pm$  standard deviation. Means with different superscript down the column are statistically significant (p<0.05). Normal C: normal control, Negative C: negative control, 5%W: 5% *Citrullus lanatus* whole fruit, 5% P: 5% *Citrullus lanatus* pulp

#### Discussion

The result of this study suggests the protective effect of *C. lanatus* supplementation on oxidative stress and haematological indices in rats. Acetaminophen causes hepatic toxicity, which could lead to oxidative stress if left uncontrolled (Larsen and Wendon, 2014). Expectedly, the groups treated with 2g/kg acetaminophen exhibited significant (p<0.05) reduction in the levels of antioxidant enzymes and haematological indices except Platelets level. The insignificant(p<0.05) decrease in the platelets counts might be due to the duration (12 hours) between induction of oxidative stress and sacrifice; a longer time interval could probably result to a more significant effect.

C. lanatus is rich in various phytochemicals of great health benefits (Zamuz et al., 2021, Okolo et al., 2025). Findings from this study showed that C. lanatus supplementation significantly (p<0.05) increased the level of antioxidants in rats treated with acetaminophen. Many works have suggested the possible antioxidant effect of different parts of C. lanatus (Asghar et al., 2013, Oyenihi et al., 2016, Akintunde et al., 2021). However, results from this study show that the group supplemented with 5% pulp of C.lanatus had more increase in antioxidant enzymes compared to the group supplemented with 5% whole fruit of C. lanatus. This may be due to the levels of phytochemicals in the pulp. Reports showed that flavonoids are more concentrated in the pulp (Ibra et al., 2017). There may also be some presence of bioactive compounds impairing the antioxidant capability of the whole fruit of C. lanatus.

Oxidative stress can cause haemolysis, consequently, causing alterations in haematological indices (Biswas et al., 2005). Findings from this study showed that there was no significant difference in the haematological indices across all groups during the feeding and supplementation phase. However, upon treatment with acetaminophen, C. lanatus exhibited a protective effect in the haematological parameters. Groups supplemented with 5% pulp and 5% whole fruit of C. lanatus showed significantly (p<0.05) higher PCV level, RBC count and haemoglobin concentration. A significant (p<0.05) decrease was observed in the WBC count while there was no difference (p<0.05) in the platelet count. This report suggests that C. lanatus possess some haematinic effects (Akintunde et al., 2020). Interestingly, the effects on haematological indices were more profound in the group supplemented with 5% whole fruit and may be due to the phytochemical composition of the whole fruit. Previous report showed that the whole fruit of C.lanatus contained significantly higher levels of tannin, phytate and alkaloids (Okolo *et al.*, 2025). However, more work is required to elucidate the active components of *C. lanatus* responsible for these effects.

#### CONCLUSION

In conclusion, based on the level of enzymatic antioxidants, this study suggests that while supplementation with the pulp of *C. lanatus* may prevent hepatic acetaminophen toxicity by improving hepatic enzymatic antioxidant level, supplementation with whole fruit of *C. lanatus* may improve haematological indices.

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