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Full Length Research Article

HAEMATOLOGICAL AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *CITRULLUS LANATUS* LEAVES IN ACETAMINOPHEN-INDUCED OXIDATIVE STRESS

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ABSTRACT
Acetaminophen is a commonly used analgesic and antipyretic; however, overdose could cause oxidative stress related hepato-toxicity. The present study investigated the haematological and antioxidant effects of *C.lanatus* methanolic leaf extract in rats induced with acetaminophen. Rats were divided into five groups. Normal control group was not induced and not treated, negative control group was induced with acetaminophen and untreated; while group 3, 4 and 5 were induced with acetaminophen and treated with methanolic extracts of *C. lanatus* (at 250 mg/kg, 500 mg/kg and 1000 mg/kg of body weight respectively). Malondialdehyde, SOD, glutathione (GSH) and catalase in hepatic supernatant were determined. While PCV, haemoglobin concentration, white blood cell and red blood cell count were determined to elucidate the haematological effects. The results were analyzed using ANOVA and LSD test (SPSS V.20). The results showed that the administration of methanol extract of *C. lanatus* significantly ($p < 0.05$) increased catalase and GSH levels. The reduction in malondialdehyde level was not statistically significant while; SOD levels remained unchanged across all groups. A significant increase in PCV and haemoglobin concentration, red blood cells count and white blood cell count were observed in the treatment groups. This study demonstrated the antioxidant and haematological potential of *C. lanatus* leaves.

Keywords: Antioxidant, *Citrullus lanatus,* Oxidative stress, Haematological, Acetaminophen.

INTRODUCTION

Acetaminophen is the most common over-the-counter medicines used for its analgesic and anti-inflammatory effect (Lee, 2017). It is clinically safe at therapeutic doses; however, an overdose can cause oxidative stress and oxidative stress-related damages (Larsen *et al*., 2014; Ommati *et al*., 2017). Oxidative stress occurs as a result of an overproduction of reactive oxygen species (ROS) and the inability of the defense system to mop up these reactive intermediates. Hence, free radicals may cause cellular damages (Chikezie *et al*., 2015). Destruction caused by free radical include lipid peroxidation, protein oxidation, DNA strand breaks, reduced endogenous enzymes activities and alterations in cell membrane fluidity (Halliwell, 2011; Zhang *et al*., 2020).

Interestingly, there has been a global shift to the use of medicinal plants in managing many pathologies. Plants have potential for treating and preventing complications related to oxidative stress (Ramana *et al*., 2014; Messaoudi *et al.,* 2019; Tian *et al*., 2023). The efficacy of plants in managing pathologies may be due to the presence of secondary metabolites (Choudary *et al*., 2021). One of such plants is *Citrullus lanatus* (in the family, Cucurbitaceae).

Citrullus lanatus is popularly known as water melon. It is an edible fruit that belongs to the family Cucurbitaceae (Zia *et al.,* 2021). It is of therapeutic interest due to the presence of secondary metabolites in all the parts of the plant (Erhirhie and Ekene, 2013). Studies showed that Cucurbitaceae plants contain triterpenes, sterols, cucurbitacin and alkaloids. These phytochemicals are known for their therapeutic effects. Furthermore, leaves of water melon were reported to have analgesic, anti-inflammatory and anti-microbial effects (Rahman *et al*., 2013; Hameed *et al.,* 2020).

Citrulus lanatus leaves were also reported to have an *in vitro* antioxidative effect (Aruna *et al*., 2014). Therefore, the leaves of *C. lanatus* may be effective in reversing oxidative stress and complications associated with it. In view of this, the present study evaluated the haematological and antioxidant effects of methanolic extract of *Citrullus lanatus* leaves in rats with acetaminophen-induced oxidative stress.

MATERIALS AND METHODS

Chemicals and Reagents

Chloroform, sodium chloride, methanol trichloroacetic acid (TCA) and hydrochloric acid (HCl) were obtained from Henan Tianfu Chemical Company Limited, Zhengzhou, China. Sulphosalicyclic acid and 5, 5-dithiobis-2-nitrobenzoic acid were procured from Kem Light Laboratory Limited, India. Adrenaline, hydrogen peroxide, sodium bicarbonate, sodium carbonate, sodium azide, potassium hydrogen phosphate and potassium dihydrogen phosphate were purchased from Sigma Aldrich Company, USA. Acetaminophen (Emzor) was purchased from a reputable pharmacy.

Collection of Plant Material

The leaves of *Citrullus lanatus* were obtained from Sabon Layin Kaura, Dandume Local Government Area of Katsina state, Nigeria. It was authenticated at the herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria with a voucher specimen number 1266.

Preparation of Plant Extract

The leaves of *Citrullus lanatus* obtained were washed and shade dried after which it was ground into powder using pestle and mortar. The powdered leaves (100g) were packed well in methanol for 48 hours. Thereafter, it was filtered, the filtrate was left to evaporate and stored at room temperature prior to usage.

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Experimental Animals
Twenty-five (25) adult male Wister rats weighing 120g-150g were obtained from the animal house of the 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. The rats were allowed to acclimatize for two weeks prior to the study. The study protocol was done according to the approved guidelines by Ahmadu Bello University Zaria experimental animal ethics committee.

Preliminary studies (Determination of LD50)
The method used to determine acute toxicity was that described by Lorke (1983). The study was conducted in two phases. In the first phase, three groups of three rats each were administered with the extract at respective oral doses of 10mg, 100mg, and 1000mg per kg body weight. The rats were observed for signs of toxicity and possible deaths for 24 hours. In the second phase, another three groups of one rat each were administered respective doses of 1500, 2900 and 5000mg per kg body weight of the extract. They were equally monitored as in phase one for toxicity signs and deaths. No death and toxicity were observed in all the doses examined.

Experimental Protocol
The rats were divided randomly into five groups (five rats per group). Group one was labeled normal control (that is, group of rats not induced with oxidative stress and therefore not treated).
Group two was labeled negative control (that is, group of rats induced with oxidative stress and not treated). Group three, four and five were labeled experimental groups (that is, group of rats induced with oxidative stress and treated with different concentration of the methanol extract). Groups 3, 4 and 5 were orally administered the extract with a daily dose of 250, 500 and 1000mg/kg respectively for 7 days.

Induction of Oxidative Stress and Collection of Samples
On 8th day, the animals were administered with a single dose of acetaminophen (2 g/kg body weight) orally. Forty-eight hours after administration of acetaminophen, the animals were anaesthetized using chloroform. Blood sample was taken through cardiac puncture. The animals were then dissected; the liver was removed and homogenized in ice cold phosphate buffer (10% w/v; pH 7.4) to prepare the homogenate. The homogenate was centrifuged at 3000 rpm for 10 min at 4°C and the supernatant was used for further studies.

Determination of Haematological Parameters
Three (3) ml of blood sample was collected by cardiac puncture into EDTA tube. Then the red blood cell count (RBC), hemoglobin (Hb) concentration, packed cell volume (PCV) and White blood cell count were determined using auto-hematological analyzer (Beckman Coulter, USA)

Evaluation of Hepatic Oxidative Stress Markers
Lipid peroxidation was determined as thiobarbituric acid reactive substances according to Ohkawa *et al*., (1979) with slight modification by using trichloroacetic acid 15% (TCA) and thiobarbituric acid 0.67% (TBA) and GSH was measured according to Ellman (1959). Catalase activity was measured by the method Aebi (1974). Superoxide dismutase (SOD) was determined according to the method described by Fridovich, (1979); while total protein was determined using Biuret method.

Statistical Analysis
Significant differences were determined using the One-Way Analysis of Variance (ANOVA) followed by the LSD post hoc tests. A p-value ≤0.05 was considered statistically significant. Results obtained were presented as mean ± standard deviation (SD).

RESULTS

The effects of methanolic extracts of *C. lanatus* leaves on the haematological parameters of rats with acetaminophen-induced oxidative stress
Table 1 shows the result for the effect of methanolic leaves extract of *C,lanatus* on haemoglobin concentration. There was a significant ($p < 0.05$) reduction in the haemoglobin concentration of the untreated negative control group when compared to the normal control groups. However, the extract caused a significant ($p < 0.05$) increase in the haemoglobin concentration with the group treated with 250mg/kg of extract showing the most remarkable increase in haemoglobin concentration.

Table 1: Effect of methanol extract of *Citrullus lanatus* leaves on haemoglobin concentration of acetaminophen induced rats

Groups	0	Days 7	Terminal
Normal Control	14.28±1.02^{b}	14.90±0.04^{d}	15.10±0.18^{e}
Negative Control	14.08±0.17^{ab}	14.08±0.15^{b}	10.53±0.46^{a}
250 mg/kg body wt	13.43±0.18^{a}	13.35±0.13^{a}	14.63±0.10^{d}
500 mg/kg body wt	14.40±0.19^{b}	14.13±0.12^{b}	13.30±0.08^{c}
1000 mg/kg body wt	14.20±0.14^{b}	14.30±0.08^{c}	12.52±0.05^{b}

$^{a-d}$values with different superscript alphabets along a column are significantly ($p < 0.05$) different from each other.

There was no difference in the PCV level across all groups before induction with acetaminophen. However, after induction with acetaminophen, the PCV significantly reduced ($p < 0.05$) in the negative control group but groups treated with the extract showed a significant increase in their PCV levels. The increase in PCV level was more significant in the group treated with 250m/kg dose of the extract as presented in table 2.

Table 2: Effect of methanol extract of *Citrullus lanatus* leaves on packed cell volume (PCV) of acetaminophen-induced rats

Groups n=5	0	Days 7	Terminal
Normal control	43.50±3.12^{a}	44.75±1.50^{b}	46.40±1.29^{d}
Negative control	42.50±2.08^{a}	42.50±2.08^{ab}	32.20 ±1.41^{a}
250 mg/kg	42.25±2.22^{a}	41.50±1.29^{a}	44.01±0.82^{c}
500 mg/kg	42.75±1.71^{a}	42.75±1.71^{ab}	40.75±0.50^{b}
1000 mg/kg	42.50±2.08^{a}	43.25±0.96^{ab}	39.20±0.81^{b}

$^{a-d}$values with different superscript alphabets along a column are significantly ($p < 0.05$) different from each other.

Table 3 showed the effect of the extract on white blood cell count. While there was no significant difference ($p < 0.05$) in the white blood cell count of the negative control group when compared with the normal control group, a significant increase in white blood cell count was observed in the group treated with 500mg/kg of the extract.

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Table 3: Effect of methanol extract of *Citrullus lanatus* leaves on white blood cell count of acetaminophen-induced rats

Groups n=5	0	Days 7	Terminal
Normal control	4.30±0.32^{a}	4.15±0.13^{b}	4.33±0.17^{bc}
Negative control	4.23±0.22^{a}	4.20±0.14^{b}	4.18±0.10^{a}
250 mg/kg	4.48±0.13^{a}	4.45±0.12^{c}	4.45±0.13^{d}
500 mg/kg	4.41±0.18^{a}	4.30±0.08^{bc}	4.55±0.06^{d}
1000 mg/kg	4.23±0.22^{a}	3.78±0.10^{a}	4.38±0.10^{cd}

$^{a-d}$values with different superscript alphabets along a column are significantly (p< 0.05) different from each other.

Furthermore, the methanolic extract of *C. lanatus* significantly increase the red blood cell count post induction with oxidative stress in the treatment groups when compared to the negative control group. This effect was more pronounced in the group treated with 500mg/kg of the extract as presented in Table 4.

Table 4: Effect of methanol extract of *Citrullus lanatus* leaves on red blood cell count of acetaminophen induced rats

Groups	0	Days 7	Terminal
Normal Control	5.30±0.32^{a}	5.35±0.26^{a}	6.05±0.19^{b}
Negative control	5.28±0.17^{a}	5.23±0.10^{a}	5.27±0.09^{a}
250 mg/kg body wt	5.65±0.13^{b}	5.83±0.09^{c}	6.43±0.10^{c}
500 mg/kg body wt	5.30±0.18^{a}	5.68±0.08^{b}	6.60±0.08^{d}
1000 mg/kg body wt	5.55±0.13^{ab}	5.75±0.13^{b}	6.35±0.06^{c}

$^{a-d}$values with different superscript alphabets along a column are significantly (p< 0.05) different from each other.

The effects of methanolic leaves extract of *C. lanatus* on hepatic oxidative stress markers

Compared to the normal control group, the lipid peroxidation level in the negative control group was significantly ($p < 0.05$) elevated but the reduction observed in the treatment groups was not statistically significant ($p < 0.05$). Furthermore, the glutathione, catalase and SOD levels in the liver were significantly ($p < 0.05$) reduced in the negative control group compared to the normal group. However, while there was a significant ($p < 0.05$) increase in the catalase levels in a dose-dependent manner in the treatment groups compared to the compared to the negative control group, there was no statistical difference in the superoxide dismutase activity across all groups. A significant ($p < 0.05$) increase in the GSH level was also observed in the treatment groups compared to the negative control group (Table 5).

Table 5: Effect of methanol extract of *Citrullus lanatus* leaves on oxidative parameters of the liver of acetaminophen induced rats

Group n=5	CAT (µmol/ min/mg protein)	SOD (% inhibition)	MDA (nmol/mg protein)	GSH (µg/ml)
Normal Control	1.14±0.33^{b}	59.65±15.42^{a}	2163.95±132.74^{a}	83.82±2.45^{b}
Neg. C	0.48±0.30^{a}	48.66±9.26^{a}	3946.25±968.81^{b}	78.01±5.72^{a}
250mg/kg	0.92±0.36^{ab}	73.26±17.09^{a}	2291.47±1050.47^{ab}	86.4±4.30^{b}
500mg/kg	1.49±0.47^{b}	68.89±10.68^{a}	2841.76±961.37^{ab}	87.26±2.09^{b}
1000mg/kg	1.61±0.52^{b}	57.94±23.04^{a}	2444.90±1553.37^{ab}	83.45±3.89^{b}

$^{a-d}$values with different superscript alphabets along a column are significantly (p< 0.05) different from each other. Neg c- negative control group (induced with acetaminophen and untreated with extracts)

DISCUSSION

Acetaminophen is a commonly used medicine for its analgesic and anti-inflammatory effects. However, overdose may cause oxidative stress due to the increased production of free radicals (Li *et al*., 2015). With the increased interest in the use of plants for medicinal/therapeutic purposes, this study evaluated the haematological and antioxidant effects of *C. lanatus* in rats induced with oxidative stress. The possible effects of methanolic extract of *C. lanatus* in the dosage range 10-1000 mg/kg were evaluated, which confirmed the safety of fraction at this dosage range.

Results showed that except for superoxide dismutase, the methanolic extract of *C. lanatus* leaves exhibited significant ($p \leq 0.05$) antioxidative effect. This could be attributed to the presence of secondary metabolites in the leaves of *C. lanatus*. Aruna *et al*., 2014 reported that the methanolic extract of *C. lanatus* contain bioactive compounds such as flavonoid, phenolic compound, tannin, triterpenes, sterols, alkaloids and vitamins. The antioxidative property was attributed to its vitamin-C, polyphenolic, tannins and flavonoid content.

The increase in the level of lipid peroxidation in the liver of the animals in the negative control group suggests excessive formation of free radicals and activation of lipid peroxidation system. However, results showed that reduction in lipid peroxidation in groups treated with the extracts was not statistically significant ($p \leq 0.05$). This may be attributed to the reduced level of glycosides and absence of anthraquinone in the plant extract (Wapa *et al*., 2021). Glycosides have been reported to demonstrate good antioxidant potential by inhibiting lipid peroxidation (Mamta *et al*., 2013). Overall, the leaves of *C. lanatus* exhibited considerable antioxidative effects.

Erythrocytes are primary targets of oxidative stress due to its function as an oxygen carrying cell (Bissinger *et al*., 2018). The impact of free radicals on the cell membrane could cause damage to the red blood cell thereby resulting in red blood cell dysfunction, platelet destruction and tissue injury which may affect the functions of the blood cells (Comazzi *et al*., 2004). The present study showed that there was a significant ($p \leq 0.05$) increase in the haemoglobin level, packed cell volume, RBC count and WBC count of groups treated with *C. lanatus* extract when compared to the untreated group. Haematological parameters are often predictive of the toxicity of plants (Mishra *et al*., 2012).

The results of this study showed that the phytochemicals present in *C. lanatus* did not illicit any negative haematological effects. The increase in the haematological parameters may be attributed to the antioxidative capability of methanolic extract of *C. lanatus* as shown in the results of this study. Overall, our findings showed that methanolic extract of *C. lanatus* may have haematinic effect. However, more studies are still required to evaluate the biological properties of the active constituents of *C. lanatus* leaves.

Conclusion

In conclusion, this data suggests that methanolic extract of C. *lanatus* leaves may prevent hepatic acetaminophen toxicity by improving hepatic tissue oxidant/antioxidant balance. In addition, the extract may have haematinic effects.

Conflict of Interest

The authors declare no conflict of interest in the study.

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