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Preliminary Study on the Effect of Methanolic Extract of Watermelon (C*itrullus lanatus*) Rind on Prednisolone Suppressed Immunity in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all the authors. Author NYW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MSK, GO and OA managed the analyses of the study. Author EEK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Citrullus lanatus thumb (Cucurbitaceae) commonly called watermelon is widely consumed in this part of the world as food and medicine. This study was carried out to examine the effect of methanolic extract of watermelon (*Citrullus lanatus*) rind on prednisolone suppressed immunity in male wistar rats. A total of 20 male wistar rats weighing 150-294g were used in 4 groups with five rats each. Group 1, the control group was given distilled water and feed, Group 2 was given 200 mg/kg body weight of methanolic extract of watermelon rind, Group 3 rats were given 2.5 mg/kg body weight of prednisolone and Group 4 rats were given 2.5 mg/kg body weight of prednisolone

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and 200 mg/kg body weight of methanolic extract of watermelon rind. Prednisolone and the methanolic extract of watermelon rind were administered orally for a period of 30 days. Blood samples were collected by cardio puncture from the rats for white blood cell (WBC), lymphocyte, and granulocyte and monocyte counts at the end of the experiment. The data were statistically analysed using one-way ANOVA (Analysis of variance). Data were considered significant at $p<0.05$. The results obtained showed that methanolic extract of watermelon rind caused a significant increasen in immune function of rats when compared with the control and immune suppressed rats.

Keywords: Watermelon; prednisolone; immunity; granulocyte; lymphocyte.

1. INTRODUCTION

Fruits and vegetables are known to contain a variety of natural bioactive compounds [1] such as flavonoids, anthocyanins, vitamin C and E, phenolic compounds, dietary fibre, and carotenoids [2]. Consumption of fruits and vegetables in regular diet provides several health benefits.

Watermelon, *Citrullus lanatus* (Thunb), from the family of cucumber (cucurbitacea), is a large, oval, round or oblong tropical fruit [3]. It is a notable horticultural crop cultivated widely for its delicious fruits. Every aspect of the fruit has nutritional value, including the rind, peel and the seeds. Asian countries contribute approximately 81% of total production of watermelon worldwide [4]. According to the Food and Agricultural Organization of the United Nations, a cultivation area of 3.2 million hectares was employed for the production of 103 million tons of watermelon worldwide in 2018 [4]. The watermelon fruits are used for the preparation of smoothies, jams, sauces, candies, and juices. Watermelon serves as a vital natural source of L-citrulline (0.9 to 5 mg/kg of fresh fruit) [5]. The refreshing taste, high water content, and its attractive colors ranging from red, yellow, and pink increases the consumption of watermelon. The diverse colors of watermelon are due to the presence of carotenoids especially, lycopene and β-carotene [5]. In particular, watermelon can be considered as an excellent functional food due to its rich lycopene, vitamin A, vitamin C contents and antioxidant potentials [6,7].The therapeutic effect of watermelon has been reported and has been ascribed to antioxidant compounds [8,9].

Watermelon rind is the outer part (exocarp) of a watermelon fruit and is usually light green or white in color. It is usually discarded, applied to feeds or fertilizer but they are also edible and sometimes used as vegetable. Pickled watermelon rind is also commonly consumed in the Southern US, Russia, Ukraine, Romania and Bulgaria [9]. It contains phenol and flavonoid compound which protect the human body from free radicals [10]. It contains large amounts of beta carotene, which is an antioxidant [11] that can help reduce the severity of asthma attacks, and reduce the inflammation in osteoarthritis and rheumatoid arthritis. It is a significant source of lycopene which plays a prominent role in the treatment and management of ailments such as cancer and cardiovascular disease [10]. It also contains citrulline, an amino acid that helps dilate blood vessels to improve circulation [11].

Prednisolone is a corticosteroid drug with predominant glucocorticoid and low mineralocorticoid activity, making it useful for the treatment of a wide range of inflammatory and auto-immune conditions [12].

The objective of this research was to investigate the effect of watermelon rind on prednisolone suppressed immunity in male wistar rats.

2. MATERIALS AND METHODS

2.1 Animal Grouping

Male Wistar rats (20 in number) weighing 150-294g were used in the study. The animals were bought from Zoological Garden, University of Nigeria, Nsukka, Enugu State and taken to animal house of Madonna University, Elele, kept in cages and allowed to acclimatize for 2 weeks after which they were randomly selected into 4 different groups; group 1, 2, 3 and 4.

These animals were fed with standard rat feed (Guinea feeds, with composition; Protein 14.5%, Fat 4.8%, Fiber 7.2%, Calcium 0.8%, Phosphorus 0.62%, Sodium 0.15% and metabolizable energy 2,300 cal/kg, and water ad libitum).

2.2 Phytochemical Analysis of Watermelon Rind

2.2.1 Alkaloids determination

0.2 ml dilution of each extract was measured into a 250 ml beaker and 50 ml of 10% acetic acid in ethanol was added and allowed to stand for some

minutes. This was filtered and the extract was concentrated on a water bath for one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid which was dried and weighed.

Table 1. Showing phytochemical analysis of watermelon rind

Phytochemicals	Watermelon rind
Saponin	+
Tannin	–
Anthraquinine	+
Flavonoids	–
Steroids	–
Terpenoids	+
Alkaloids	+
Oils	+
Alkaloids	+
Keller killiani	+
Legal test	+

2.2.2 Saponin determination

0.2 ml dilution of each extracts was placed into a test tube with propan-2-ol for a day in the ratio 1:10 for each sample, 2 ml of 40% solution of magnesium sulphate ($MgSO_4$) solution was added. The mixture obtained was filtered through No 1 Whatman filter paper obtaining a clear colorless solution. 5% $FeCl_3$ solution was observed and the absorbance was read at 380 nm and saponin content was calculated accordingly.

2.2.3 Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Minotti et al. (1987). Each extract was measured into three test tubes in the range of 50, 100, and 200 µL and each was mixed with 500 µL of methanol. Water was added to mark up to 200 µml. 50 µml 10% $AlCl_3$ followed by 50 µL of 1M potassium acetate and 1400 µL water was added and allowed to incubate at room temperature for 30 mins. The absorbance of the reaction mixture was measured at 415 nm; the total flavonoid content was subsequently calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

2.3 Experimental Design

A total of 20 adult male albino wistar rats weighing 150 to 294 g were used for the experiment. All the rats were kept in an average room temperature with 12 hours natural light and kept in the animal house of the Faculty of Basic Medical Sciences, Madonna University, Elele, Rivers State.

The rats were allowed to acclimatize for two weeks and their health status was closely monitored in a clear room inside a wooden cage. They were fed with normal rat feed and water. All the male albino wistar rats were divided into 4 groups.

Table 2. Showing tabulated presentation of the experimental design

Groups	Number of rats	Quantity of drug administered (prednisolone)	Quantity of extract administered (*Citrulus lanatus*)
Group 1	5	-	Normal feed and water
Group 2	5	-	200 mg/kg
Group 3	5	2.5 mg/kg	Normal feed and water
Group 4	5	2.5 mg/kg	200 mg/kg

2.4 Administration of Extract

The watermelon rind extract was administered through oral route using 5 ml syringe without needle.

$$\text{Dose in ml} = \frac{\text{Dose in mg/kg x weight of rat}}{\text{Dose in ml/kg}}$$

$$= \frac{200 \text{ x weight of rat}}{100}$$

2.5 Administration of Prednisolone

Prednisolone was administered through intra-peritoneal route and care was taken not to cause damage of the organs in that region. The dosage of the drug administered to each rat was calculated based on the weight of each rat. It was subsequently increased or decreased weekly depending on the weight of the rats.

Table 3. The effect of methanolic extract of watermelon rind and prednisolone on white blood cell, monocyte and granulocyte

Groups	WBC × 10^{-9}/L	Monocyte × 10^{-9}/L	Granulocyte × 10^{-9}
Group 1	18.02±3.34	1.71±0.16	4.35±0.30
Group 2	100.26±9.30*	4.09±0.86*	97.07±8.16*
Group 3	13.24±0.92	1.17±0.11	1.87±0.21
Group 4	92.19±5.12*	4.48±0.87*	72.14±7.99*

Table 4. showed the effect of methanolic extract of Watermelon rind and prednisolone on lymphocyte, monocyte and granulocyte

Groups	Lymphocyte %	Monocyte %	Granulocyte %
Control	56.97±5.93	6.83±0.41	31.63±4.76
High dose	79.87±0.20*	11.05±1.36*	86.34±0.56*
Immunity	46.16±1.88*	4.53±.0.96*	14.33±1.10*
Immunity + high dose	71.70±4.09*	8.74±0.71*	76.44±4.83*

Values are expressed in mean ± SEM ($p<0.05$)

$$\text{Dose in ml} = \frac{\text{Dose in mg/kg x weight of rat}}{\text{Dose in mg/ml}} = \frac{2.5 \text{ x weight of rat}}{0.5}$$

2.6 Monitoring of the Animals

All the animals were constantly monitored on a daily basis and the rats were provided with adequate feed and water, and were maintained in a hygienic environment.

2.7 Collection of Blood Samples

At the end of four weeks, the rats were euthanised 24 hours after the last dose. Five milliliters of blood was collected via cardiac puncture. The blood samples were analysed in the chemical pathology laboratory of Madonna University Teaching Hospital, Elele, Rivers State.

2.8 Statistical Analysis

The data were expressed as mean Standard Error of Mean (SEM). The statistical evaluation of data was performed by using one-way ANOVA (Analysis of variance). Data were considered significant at $p<0.05$.

3. RESULTS and DISCUSSION

Watermelon is known to boost the immune system just like other fruits. The result showed a significant increase ($p>0.05$) in the WBC, Monocyte, Granulocyte and Lymphocyte of rats administered watermelon rind extract when compared to the control group. This increase may be as a result of the presence of some phytochemicals like saponins which according to studies have been found to have stimulatory effects on immunity [13,14] and monocyte proliferation [15,16].

The increase observed in White blood cell (WBC), Monocyte, Granulocyte and lymphocyte of rats administered prednisolone with watermelon rind extract when compared to control group supports the study that watermelon rind help boost immunity because of its lycopene content [10].

Finally, a decrease $p<0.05$ in White blood cell (WBC), Monocyte, Granulocyte and Lymphocyte of rats administered only prednisolone. The decrease could be because prednisolone is a glucocorticoid, and glucocorticoids suppress the immune system of the body by decreasing the number of circulating T lymphocytes. This is as a result of the suppressing effect of glucocorticoids on lymphoid tissue and thymus.

4. CONCLUSION

The result of the present study has shown that administration of prednisolone suppresses the immune system whereas administration of *Citrullus lanatus* rind extract boosts immunity. This supports the nutritional and health benefit of watermelon rind.

CONSENT

It's not applicable.

ETHICAL APPROVAL

All the animals were under standard managemental conditions according to the rules and regulations of the institute of Animal Ethics Committee (IAEC).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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