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Impact of oral administration of bromelain-derived bitter gourd seed protein hydrolysate on some Hematological parameters in healthy Wister albino rats

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Abstract

Momordica charantia has long been abused in northern Nigeria for treating diabetes mellitus, cancer, irregular stomach, fever, and birth control. It has been noted that the majority of those affected are those living in rural areas where access to modern medical facilities is limited, with 80% of them depending on traditional medicine. This study was aimed to evaluate the effect of oral administration of bromelain-derived better gourd seed protein hydrolysate on some hematological parameters in healthy wister albino rats. Bromelain-derived better gourd seed protein hydrolysate was produced by hydrolysis of better gourd seed using bromelain enzyme. Fifty (50) healthy wister albino rats were grouped according to their body weights. Normal control group was administered orally with distilled water daily for twenty-eight (28) days. Test groups T100, T200, T400 and T800 were administered orally with 100, 200, 400 and 800 mg/kg body weight of bromelain-derived better gourd seed protein hydrolysate for twenty-eight (28) days respectively. The rats were humanely sacrificed, blood samples were collected through cervical dislocation, for evaluation of some hematological parameters (red blood cell (RBC), white blood cell (WBC), hemoglobin (HB), packed cell volume (PCV), platelet (PLT), cholesterol (CHOL), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), urea, creatinine, alanine aminotransferases (ALAT) and aspartate aminotransferases (ASAT). One-way ANOVA was used to compare the mean of some of the hematological parameters. It was observed that bromelain-derived better gourd seed protein hydrolysate elevates the white blood cell (WBC) count and packed cell volume (PCV) when compared to the normal control group at doses of 400 and 800 mg/kg body weight. There was an increase in the value of alanine and aspartate aminotransferases (ALAT and ASAT respectively) in groups treated with higher dose of 800 mg/kg body weight bromelain-derived bitter gourd seed protein hydrolysate when compared to the normal control group. It was also observed that the red blood cell count (RBC), hemoglobin (HB), platelet (PLT), cholesterol (CHOL), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), urea, and creatinine of the groups treated with bromelain-derived better gourd seed protein hydrolysate showed no significant changes in values when compared to the normal control group. The bromelain-derived better gourd seed protein hydrolysate at high dose has potential in elevating some of the hematological parameters (WBC, PCV, ALAT, and ASAT) which might have toxic effect on the liver and hematopoietic system.

Keywords: Medicinal plant; *Momordica charantia*; Bromelain-derived bitter gourd seed protein hydrolysate; Hematological parameters; Healthy Wister albino rats.

1. Introduction

Nature consistently provides a clear indication of the notable occurrences of coexistence. Natural remedies for illnesses in humans are based on substances found in plants, animals, and minerals. The popularity of medicinal plants is growing, and demand for them is high right now. Plants unquestionably contribute significantly to ecosystems by offering vital services. Humans and other living things cannot exist as they ought to in the absence of plants. In any case, herbs especially medicinal ones have long served as a general indicator of the health of the ecosystem. Medicinal plants are

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any of the many types of plants that provide therapeutic qualities. Different kinds of seeds, roots, leaves, fruit, skin, flowers, or even the entire plant are among the parts of medicinal plants that might be employed. The active compounds in most parts of the medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents. In the body of these plants, certain materials are produced and stored that are referred to as active compounds (substances), which have physiological effects on the living organisms (Sofowora *et al.,* 2013).

The primary source of medical supplies used by humans to maintain health and treat illnesses is raw plant material. Because of their various qualities, especially their synergistic effects, medicinal plants are employed in medicine. It is possible for the plant's constituents to interact with one another, which might be advantageous to both, detrimental to one, or erase the negative effects of both. Complicated diseases like cancer can be significantly improved by substances derived from plants. Another characteristic of plant components is their capacity to halt the spread of specific illnesses. The toxicity and adverse effects of conventional and allopathic medicines have also been important factors in the sudden increase in population demands and increase in the number of herbal drug manufactures as well as a reduction in the use of chemical drugs (Jamshidi-Kia *et al.,* 2017).

The primary indicators of an organism's physiological, pathogenic, and nutritional status are blood parameters. Deviations from standard values in blood constituents can provide insight into an animal's metabolic condition. One of the most vulnerable areas to harmful substances, it serves as a crucial gauge of health and disease in both humans and smaller animals (Mukinda and Syce, 2007). These parameters include red blood cells count, hemoglobin concentration, packed cell volume, platelets count, total and differential white blood cells count and erythrocyte sedimentation rate. A complete blood count (CBC) is a common blood test that provides detailed information about the three types of cells in the blood: Red Blood Cells (RBS), White Blood Cells (WBC), Hemoglobin (Hb) and Platelets. Each type of blood cell plays an important role in the body's normal function.

Momordica charantia is a plant that is used as a food and natural medicine. It is also referred to as bitter melon and bitter guard. *Momordica*, the scientific name, comes from the Latin word "to bite," which describes the sharp edges of the leaves. Every portion of the plant, including the fruits, has a bitter substance called momordicinso, which has an extremely bitter flavor. The plant grows in tropical regions such as India, China, America Malaya, Bangladesh, tropical Africa, Thailand, Middle East. Several biologically active phytochemicals, such as proteins, triterpens, saponins, flavonoids, steroids, alkaloids, and acids, are present in *Momordica charantia.* The plant is beneficial for its anti-tumorous, anti-fungal, anti-parasitic, anti-cancer, antiviral, anti-fertility, anti-bacterial and hypoglycaemic properties due to the presence of numerous phytochemicals. In traditional medication, fruits and leaves are used to cure several diseases like: gout, rheumatism, colic, worms, illness of liver and spleen. *Momordica charantia* has hypoglycemic properties because it includes peptides and alkaloids that resemble insulin and charantin, a group of steroidal sapogenins. (Anilakumar *et al.,* 2015).

Momordica charantia has long been abused in northern Nigeria for treating diabetes mellitus, cancer, irregular stomachs, fever, and birth control. It has been noted that the majority of those affected are those living in rural areas where access to modern medical facilities is limited, with 80% of them depending on traditional medicine. *Momordica charantia* has been abused extensively, and little is known about its adverse consequences. Given that not all of the plant extract's constituents are typically medicinal (Umar., 2017).

Protein hydrolysates and biopeptides have various biological activities such as antioxidant, antidiabetic, antibacterial, and antihypertensive, potential based on chain length, hydrophobicity, amino acid content, and sequencing (Nasri, 2017). However, there are some inadequate information's about bromelain-derived bitter gourd seed protein hydrolysate regarding the effects of bioactive peptides hydrolysate and impact of the bioactive peptides on some hematological parameters. Therefore, there is need to provide more information about the active protein(s) in *Momordica charantia* especially those of seed and evaluate their effect on some hematological parameters. This study was aimed to evaluate the impact of bromelain-derived bitter gourd seed protein hydrolysate on some hematological parameters in healthy wister rats which may be considered nontoxic and with low side effects.

2. Materials and methods

2.1. Sample preparation

Seed of *Momordica charantia* was separated from the fruits, they were washed under running tap water, and freezed dry using freeze drier. The dried seed were pulverized to a fine powder using laboratory blander. The powdered sample of *Momordica charantia* seed was then kept in air-tight polythene bag at -20 ^{0}C.

2.2. Experimental design

2.2.1. Animals and Treatment

Fifty (50) Sprague–Dawely healthy rats (130–100 g) were obtained from National Veterinary Research Institute (NVRI), Vom, Jos State, Nigeria. The animals were housed in standard cages under proper environmental conditions, feed with a commercial diet, tap water provided ad libitum and kept for 2 weeks for acclimatization (Awad et al., 2016). Animals were randomly distributed into five (5) groups of ten (10) rats each and were treated with the bromelain-derived bitter gourd seed protein hydrolysate orally for 4 weeks (28 days) in which analyses was carried out on last day of 28 days.

Table 1 Groups and doses of Bromelain-Derived Bitter Gourd Seed Protein Hydrolysate (BBGSPH) administered (mg/kg body weight)

Groups	Dosage	Number of animal
Normal control	Food/water	10
Test 1 + BBGSPH	100 mg/kg	10
Test 2 + BBGSPH	200 mg/kg	10
Test 3 + BBGSPH	400 mg/kg	10
Test 4 + BBGSPH	800 mg/kg	10

2.3. Preparation of defatted bitter gourd seed powder.

Defatting of bitter gourd seed powder to remove steroidal compounds, oily substances, and fats was carried out using method reported by (Zaharuddin *et al*., 2021). With slight modifications.

Powdered Bitter gourd seed was passed through a sieve (0.5 mm size).

The sieved Bitter gourd seed powder was defatted by mixing with petroleum ether at 1:3 (w/w) ratio and stirred continuously for 30 min at room temperature (28 °C).

The solvent was decanted, and extraction was repeated for 2 more times to achieved maximum defatting.

The defatted Bitter gourd seed powder was dried overnight under a fume hood

2.4. Protein extraction of bitter gourd seed

Protein extraction of defatted bitter gourd seed powder was carried out using method reported by (Zaharuddin *et al.,* 2021) with slight modifications to obtained Bitter gourd seed protein.

Defatted Bitter gourd seed powder was mixed with distilled water at a 1:30 ratio (w/v) and the pH was adjusted to 9 using 4 M NaOH.

The mixture was agitated for 1 hr at 80 rpm and 50 °C, followed by centrifugation at 10,000 rpm for 15 min at 4 °C.

The supernatant was collected, and the pH was adjusted to 4.5 using 0.1 M HCl.

Protein precipitate was collected after centrifugation at 10,000 rpm for 15 min at 4 °C (No 2 above), washed with water, and it was freeze dried to obtain Bitter gourd seed protein concentrate.

2.5 Protein hydrolysis of bitter gourd seed

Bitter gourd seed protein hydrolysis was carried out with bromelain enzyme using method reported by (Zaharuddin *et al.,* 2021). With slight modification. Briefly.

2.4.1. Hydrolysis

1 g of Bitter gourd protein Concentrate was mixed with 25 ml of buffer for enzyme Bromelain (50 mM acetate buffer, pH 5.0, 55 °C).

0.02g of enzyme Bromelain was added [i.e using an enzyme: substrate ratio of 1:50 (w/w)] and hydrolysis was conducted for 8 hrs.

The hydrolysed sample in the centrifuge tube containing the bitter gourd seed protein hydrolysate (BGSPH) was immersed in boiling water (100 ^{0}C) for 10 min to inactivate the enzyme.

The reaction mixture was then cooled and centrifuged at 10,000 rpm for 25 min using refrigerated centrifuge, and it was filtered through 0.22 µm syringe filter to remove any insoluble, residual material.

The supernatant containing the bromelain-derived bitter gourd seed protein hydrolysate (BGSPH) was collected, and it was used to check its effect on some haematological parameters (*In vivo* studies).

2.5. Determination of hematological parameters

All the hematological indices (Packed Cell Volume (PCV), Hemoglobin (Hb) Concentration, Red Blood Cells (RBCs), White Blood Cells [WBCs] and Platelet count [PLT]) were being assayed by using the automated hematology analyzer (Sysmex KX-21N™).

2.6. Biochemical estimation of kidney and liver function parameters

The Serum urea, creatinine, alanine aminotransferases (ALAT) and aspartate aminotransferases (ASAT) were determined in the serum samples using ChemRay 240 Semiautomated Chemistry analyzer.

2.7. Serum lipid profile assay

Serum lipid profile assay (Total cholesterol, HDL, LDL and Triglyceride) was determined in the serum samples using ChemRay 240 Semiautomated Chemistry analyzer.

2.8. Statistical analysis

All analyses were presented as mean ± SD. Data analysis was carried out using SPSS software (Version 20) and data of differences between samples was compared. ($p < 0.05$).

3. Results

Table 2 Some hematological parameters level in blood sample of healthy albino Rats after treated with Bromelain-derived Bitter Gourd Seed Protein Hydrolysate (BBGSPH)

Index	Normal Control	T100	T200	T400	T800
GLU (mmol/l)	3.0±1.73^a	3.2±0.59^a	3.7±0.10^a	3.2±0.49^a	3.1±0.17^a
RBC (U/L ×10^6)	7.35×10^6±1.40×10^{5a}	7.51×10^6±2.48×10^{5a}	7.42×10^6±3.11×10^{5a}	7.31×10^6±1.01×10^{5a}	7.41×10^6±4.36×10^{5a}
WBC (U/L ×10^3)	7.86×10^3±8.41×10^{2a}	7.63×10^3±1.54×10^{2a}	7.66×10^3±1.78×10^{3a}	8.96×10^3±1.12×10^{2b}	8.98×10^3±1.63×10^{3b}
HBG (g/dl)	14.83±0.33^a	14.16±0.33^a	14.03±0.60^a	14.30±0.25^a	14.20±0.20^a
PCV (%)	53.26±1.85^a	53.33±0.99^a	53.35±1.99^a	55.53±3.88^a	55.93±0.48^a
PLT (U/L)	6.48×10^5±6.43×10^{3a}	6.49×10^5±2.14×10^{4a}	6.48×10^5±1.23×10^{4a}	6.47×10^5±8.81×10^{2a}	6.49×10^5±5.00×10^{3a}

All values are represented as mean ± standard error of mean (SEM) of ten different replicates. Values with different superscripts down across the group are statistically different from Normal control at ($P \leq 0.05$).

The results of glucose (GLU) level in this study showed that the group treated with the bromelain-derived bitter gourd seed protein hydrolysate had non-significant elevated value of glucose level when compared to the normal control group and between the treated groups ($p \geq 0.05$). It was observed that the red blood cell (RBC) count of the groups treated with Bromelain-derived bitter gourd seed protein hydrolysate had showed no significant change in values of red blood cell (RBC) when compared to the normal control group and between the treated groups ($p \geq 0.05$). The results of white blood cell (WBC) count in this study showed that groups treated with a dose of 400 and 800 mg/kg body weight (T400 and T800 respectively) bromelain-derived bitter gourd seed protein hydrolysate had significant elevated value of the WBC count when compared to the normal control group and rats in groups treated with 100 and 200 mg/kg body weight (T100 and T200 respectively) bromelain-derived bitter gourd seed protein hydrolysate ($p \leq 0.05$). This study also revealed that there was no significant change in hemoglobin (HBG) values across the groups treated with bromelain-derived bitter gourd seed protein hydrolysate when compared to the normal control group and between the treated groups ($p \geq 0.05$). On the other hand, there was significant increase in packed cell volume (PCV) count in groups treated with a dose of 400 and 800 mg/kg body weight (T400 and T800 respectively) bromelain-derived bitter gourd seed protein hydrolysate when compared to the normal control group and rats in groups treated with 100 and 200 mg/kg body weight (T100 and T100 respectively) bromelain-derived bitter gourd seed protein hydrolysate ($p \leq 0.05$). There is no significant change in platelet (PLT) count in the rats treated with bromelain-derived bitter gourd seed protein hydrolysate when compared to the normal control group and between the treated groups ($p \geq 0.05$). (Table 2)

Table 3 Some kidney and lipid profile parameters level in serum sample of healthy albino Rats after treated with Bromelain-derived Bitter Gourd Seed Protein Hydrolysate (BBGSPH)

Index	Normal Control	T100	T200	T400	T800
ALAT (U/L)	36.33±3.28[a]	36.67±4.14[a]	36.53±3.41[a]	36.77±2.64[a]	41.67±2.33[b]
ASAT (U/L)	147.33±1.76[a]	147.63±6.76[a]	146.33±9.96[a]	147.67±8.48[a]	150.00±8.32[b]
CHOL (mmol/l)	1.51±0.67[a]	1.53±0.18[a]	1.52±0.47[a]	1.53±0.29[a]	1.54±0.12[a]
HDL (mmol/l)	0.23±0.03[a]	0.240±0.06[a]	0.25±0.09[a]	0.23±0.09[a]	0.24±0.03[a]
LDL (mmol/l)	0.40±0.03[a]	0.39±0.03[a]	0.41±0.07[a]	0.40±0.15[a]	0.42±0.07[a]
TG (mmol/l)	0.67±0.07[a]	0.66±0.09[a]	0.68±0.21[a]	0.67±0.07[a]	0.68±0.00[a]
UREA (mmol/l)	5.17±0.54[a]	5.15±1.39[a]	5.23±1.00[a]	5.27±0.35[a]	5.30±0.29[a]
CREA (mmol/l)	56.67±5.55[a]	56.63±3.53[a]	56.69±2.31[a]	56.65±2.31[a]	56.70±0.00[a]

All values are represented as mean ± standard error of mean (SEM) of ten different replicates. Values with different superscripts across the group are statistically different from Normal control at ($P \leq 0.05$).

The study revealed that there was significant increase in the value of alanine and aspartate aminotransferases (ALAT and ASAT respectively) in groups treated with a dose of 800 mg/kg body weight (T800) bromelain-derived bitter gourd seed protein hydrolysate when compared to the normal control group and rats in groups treated with 100, 200 and 400 mg/kg body weight (T100, T200 and T400 respectively) bromelain-derived bitter gourd seed protein hydrolysate ($p \leq 0.05$). The study also revealed that there was no significant change in all the lipid profile (CHOL, HDL, LDL, and TG) values across the groups treated with bromelain-derived bitter gourd seed protein hydrolysate when compared to the normal control group and between the treated groups ($p \geq 0.05$). On the other hand, there is no significant change in urea and creatinine values in the rats treated with bromelain-derived bitter gourd seed protein hydrolysate when compared to the normal control group and between the treated groups ($p \geq 0.05$). (Table 3)

4. Discussion

The degree of safety is typically determined via toxicity studies on medicinal plants or extracts from them, especially when developing new medications. Many plant-derived compounds and substances are becoming increasingly known for their medicinal effect. The toxic effects of herbal preparations on specific organs in animals and humans have been of medical concern as this is not taken into consideration by traditional healers. Organ toxicity is of concern because most herbal medicines are often used in their crude form, which contains several other constituents apart from the active ingredient (Mensah, 2019).

The hematological parameters (White Blood Cell, Red Blood Cell, Hemoglobin, packed cell volume and platelet count) did not produce any significant changes ($p \geq 0.05$) when compared to normal control group and between the treated

groups. This suggests that the bromelain-derived bitter gourd seed protein hydrolysate might have no toxic effect on the hematopoietic system and as such, will produce no gross physiological alterations in the body system. The hematopoietic system is one of the most sensitive targets for toxic compounds and important index of physiological and pathological status.

In this study, it was observed that the white blood cells (WBCs) count had showed a significant increase $p \leq 0.05$) at higher dose of 800 mg/kg body weight. The elevated levels of white blood cells (WBCs) in many conditions indicate infection, stress, inflammation, trauma, allergy or certain disease in an organ, tissue or the whole organism (Shcrier, 2010). The bromelain-derived bitter gourd seed protein hydrolysate at higher dose in this study might have caused some toxic effects on the body system of the treated rats, and consequently, the elevation of white blood cells (WBCs). This is because the white blood cells (WBC) are known to play an important role in animal body's immune system. They look for invasive fungus, bacteria, and viruses in the blood. Before an alien virus or bacteria causes illness, white blood cells (WBCs) or leukocytes identify and eliminate the invasive particle from the circulation. (Shcrier, 2007). This research is not in agreement Solomon and Akinbo 2022 on Histopathological and Haematological Changes Observed in Adult Wistar Rats Administered with Momordica charantia (Bitter Melon) Aqueous Leaf Extract and Husna *et al*., (2013) *Momordica charantia's* acute oral toxicity effects on Sprague-Dawley rats**.** But is agreement with research on effects of oral administration of *Momordica* charantia in hematological parameters of adult albino rats by Umar, (2017).

The red blood cells (RBCs) count in this study indicates no significant difference between the treated and the control groups. It is believed that when red blood cells (RBCs) count drops to lower than the normal range, it indicates anemia. Anemia has many causes including, low level of certain vitamins or iron, blood loss or due to an underlying condition (Umar 2017).

In this study, the value of alanine and aspartate aminotransferases (ALAT and ASAT respectively) was found to be elevated at higher dose which indicate that bromelain-derived bitter gourd seed protein hydrolysate at higher dose might be hepatotoxic.

The levels of CHOL, HDL, LDL and TG did not show significant changes ($p \geq 0.05$) after oral administration of bromelain-derived bitter gourd seed protein hydrolysate which is not consistent with those obtained by Zhu *et al.,* (2012) Effect of superfine grinding on antidiabetic activity of bitter melon powder. In the present study, bromelain-derived bitter gourd seed protein hydrolysate does not have significant effect on the lipid profile parameters.

Oral administration of the different doses of bromelain-derived bitter gourd seed protein hydrolysate did not lead to a significant change in the concentration of the serum urea and creatinine, suggesting that the two extracts might not be nephrotoxic. Serum urea and creatinine are also used as markers of renal functions in clinical diagnosis. (Madaki *et al.,* 2021).

5. Conclusion

In conclusion, oral administration of bromelain-derived bitter gourd seed protein hydrolysate has produced some negative effects on some hematological parameters (WBC, PCV, ALAT, and ASAT) at higher doses. Bromelain-derived bitter gourd seed protein hydrolysate causes increase in level of alanine and aspartate aminotransferases (ALAT and ASAT respectively) at a higher dose of 800 mg/kg body weight. The oral administration of bromelain-derived bitter gourd seed protein hydrolysate did not produce significant changes in red blood cell count, hemoglobin, and platelet count except at doses of 400 and 800 mg/kg body weight causes significant change in white blood cell count and packed cell volume which may possibly serve as an acceptable white blood cell and blood cell booster in an infectious/anemic condition or used for prophylactic purposes. Although the specific mechanism(s) through which the bromelain-derived bitter gourd seed protein hydrolysate enhances white blood cell and packed cell volume was not ascertained in this study, it is suggested that the bromelain-derived bitter gourd seed protein hydrolysate may have a direct effect on the body system that produces blood cells and contains constituent(s) that can interact and stimulate the formation and secretion of lymphocyte and blood cells. Oral administration of different doses of bromelain-derived bitter gourd seed protein hydrolysate did not lead to a significant change in the concentration of the serum lipid profile parameters (CHOL, HDL, LDL and TG), urea and creatinine, suggesting that the bromelain-derived bitter gourd seed protein hydrolysate might not be nephrotoxic. Serum urea and creatinine are also used as markers of renal functions in clinical diagnosis.

Recommendation

Further studies on this protein hydrolysate on molecular markers, mechanisms of action of bromelain-derived bitter gourd seed protein hydrolysate in enhancing white blood cell and packed cell volume and clinical trials to validate these data, which may lead to the development of more potent therapeutic formulations, is recommended to be carried out in the future.

Compliance with ethical standards

Disclosure of conflict of interest

The authors report no conflicts of interest.

Statement of ethical approval

This study was approved and ethical clearance was granted by Gombe State University, Nigeria, Animal care and use research ethics committee.

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