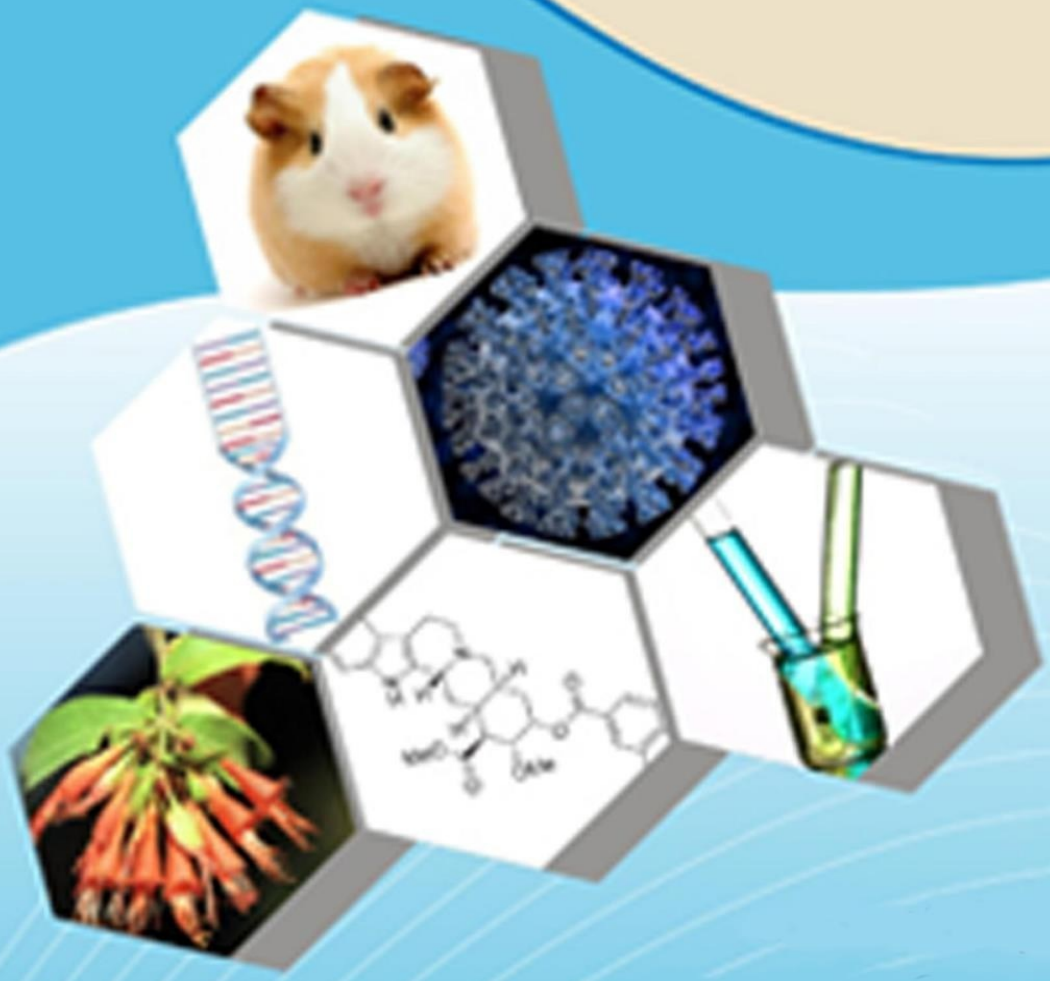
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Examining the effects on several haematological parameters in healthy Wister albino rats after oral dose of bitter gourd seed protein hydrolysate produced from bromelain

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Abstract

In northern Nigeria, momordica charantia has a long history of misuse as a treatment for diabetes, cancer, irritable bowel syndrome, high fever, and even as a method of birth control. Eighty percent of people afflicted rely on traditional medicine, and the vast majority of them reside in rural regions with little access to contemporary medical facilities. The objective of this research was to assess the impact on several hematological parameters of orally administering better gourd seed protein hydrolysate generated from bromelain to healthy wister albino rats. The hydrolysis of improved gourd seed with the help of bromelain enzyme resulted in a protein hydrolysate. We weighted fifty (50) adult wister albino rats to determine their relative health. Oral administration of distilled water was carried out daily for twenty-eight (28) days in the normal control group. For twenty-eight (28) days, the following groups were given oral doses of bromelain-derived better gourd seed protein hydrolysate: T100, T200, T400, and T800. After the rats were humanely sacrificed, blood samples were taken through cervical dislocation. These samples were analyzed for various hematological parameters, including RBC, WBC, hemoglobin, packed cell volume, platelets, cholesterol, HDL, LDL, TG, urea, creatinine, alanine aminotransferases, and aspartate aminotransferases. To compare the means of several of the hemostatic measures, one-way analysis of variance was used. At dosages of 400 and 800 mg/kg body weight, it was shown that better gourd seed protein hydrolysate, which is generated from bromelain, increases the packed cell volume (PCV) and white blood cell (WBC) count compared to the normal control group. In comparison to the control group, those given a greater dosage of bromelain-derived bitter gourd seed protein hydrolysate (800 mg/kg body weight) had elevated levels of alanine and aspartate aminotransferases (ALAT and ASAT, respectively). Additionally, when comparing the groups treated with bromelain-derived better gourd seed protein hydrolysate to the normal control group, no significant changes were found in the following laboratory parameters: red blood cell count (RBC), hemoglobin (HB), platelet count (PLT), cholesterol (CHOL), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), urea, and creatinine. A possible harmful impact on the liver and hematopoietic system might be caused by the hydrolysate of better gourd seed protein, which is generated from bromelain. At large doses, it has the ability to elevate certain hematological markers, including WBC, PCV, ALAT, and ASAT.

Hematological parameters; healthy Wister albino rats; Momordica charantia; hydrolysate of bitter gourd seed protein produced from bromelain; medicinal plant.

1. Introduction

The natural world always gives us a good idea of the noteworthy instances of cohabitation. Plant, animal, and mineral-based compounds provide the basis of natural human health therapies. Medicinal plants are now in great demand due to their rising popularity. Without a doubt, plants play an essential role in ecosystems by providing a variety of functions. Without plants, it would be very difficult for humans and other forms of life to thrive. Regardless, herbs, particularly medicinal ones, have long been used as a broad measure of ecological health. All plants, regardless of species, that have curative properties are considered medicinal plants. A wide variety of medicinal plant components, including seeds, roots, leaves, fruit, skin, flowers, and the whole plant itself, have potential for use in healthcare. Most medicinal plants include active chemicals that have therapeutic effects, either directly or indirectly. According to Sofowora et al. (2013), plants are able to create and retain molecules that are known as active compounds. These substances may affect the physiological processes of living things.

Humans rely on raw plant material as their major source for medicinal materials used for health maintenance and sickness treatment. Medicinal plants are used in medicine due to their diverse properties, particularly their synergistic effects. The plant's components may have synergistic effects, meaning they help each other out, hurt each other out, or

even cancel each other out. Substances originating from plants have the potential to greatly enhance the treatment of complex illnesses such as cancer. The ability of plant components to prevent the spread of certain diseases is another trait. The rapid rise in herbal medication production and the decline in chemical drug use may be attributed, in large part, to the toxicity and negative effects of conventional and allopathic medicines (Jamshidi-Kia et al., 2017).

Blood parameters are the main ways to tell how an organism is doing in terms of nutrition, pathology, and general health. The metabolic state of an animal may be revealed by analyzing blood components for deviations from conventional levels. It is an important indicator of human and animal health and sickness, and it is also one of the most susceptible parts of the body to toxic chemicals (Mukinda and Syce, 2007). Rhodoglobin concentration, packed cell volume, platelet count, total and differential white blood cell count, erythrocyte sedimentation rate, and red blood cell count are all examples of such measurements. One typical blood test that gives precise information on the three kinds of blood cells—RBS, WBC, and platelets—is a complete blood count (CBC). Every one of the body's blood cell types is essential for proper bodily function.

You may eat momordica charantia and utilize it as a natural remedy. It goes by other names than just bitter guard and bitter melon. The Latin term momordica means "to bite," which is a perfect description of the pointed leaves. Momordicinso is a highly bitter flavoring compound present throughout the whole plant, including the fruits. India, China, the Middle East, tropical Africa, Malaya, Bangladesh, and tropical America are all places where you may find this plant growing. Momordica charantia contains a number of phytochemicals that have biological activities, including acids, triterpens, saponins, flavonoids, steroids, alkaloids, and flavonoids. The plant's hypoglycemic, antiviral, antifungal, antiparasitic, anti-cancer, anti-fertility, antibacterial, and anti-tumor effects are a result of the many phytochemicals it contains. For many ailments, including gout, rheumatism, colic, worms, and liver and spleen illnesses, traditional medicine relies on fruits and plants. Momordica charantia contains insulin- and charantin-like peptides and alkaloids, as well as a class of steroidal sapogenins, which contribute to its hypoglycemic effects. (Source: Anilakumar et al., 2015).

Northern Nigerians have long relied on Momordica charantia for a variety of unsavory purposes, including but not limited to: diabetes, cancer, gastrointestinal issues, fever, and reproductive control. Eighty percent of people afflicted rely on traditional medicine, and the vast majority of them reside in rural regions with little access to contemporary medical facilities. Little is known about the negative effects of the widespread overuse of Momordica charantia. Since not every component of the plant extract has a therapeutic use (Umar., 2017).

Depending on factors including chain length, hydrophobicity, amino acid content, and sequencing, protein hydrolysates and biopeptides may exhibit a wide range of biological activities, including antioxidant, antidiabetic, antibacterial, and antihypertensive effects (Nasri, 2017). Unfortunately, there is a lack of sufficient data on the effects of bioactive peptide hydrolysate and the influence of these peptides on certain hematological parameters in relation to bitter gourd seed protein hydrolysate generated from bromelain. Hence, additional data about the Momordica charantia active protein(s), particularly from seed, and an assessment of their impact on certain hemostatic measures are required. This research set out to assess the effects of a potentially nontoxic and mildly side-effecting hydrolysate of bitter gourd seed protein produced from bromelain on a number of hematological parameters in otherwise healthy wister rats.

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 $\times10^6$)	$7.35\times10^6\pm1.40\times10^{5a}$	$7.51\times10^6\pm2.48\times10^{5a}$	$7.42\times10^6\pm3.11\times10^{5a}$	$7.31\times10^6\pm1.01\times10^{5a}$	$7.41\times10^6\pm4.36\times10^{5a}$
WBC (U/L $\times10^3$)	$7.86\times10^3\pm8.41\times10^{2a}$	$7.63\times10^3\pm1.54\times10^{2a}$	$7.66\times10^3\pm1.78\times10^{3a}$	$8.96\times10^3\pm1.12\times10^{2b}$	$8.98\times10^3\pm1.63\times10^{3b}$
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)	$6.48\times10^5\pm6.43\times10^{3a}$	$6.49\times10^5\pm2.14\times10^{4a}$	$6.48\times10^5\pm1.23\times10^{4a}$	$6.47\times10^5\pm8.81\times10^{2a}$	$6.49\times10^5\pm5.00\times10^{3a}$

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 ($P \leq 0.05$).

In this research, the glucose (GLU) level findings showed that the group given the bitter gourd seed protein hydrolysate generated from bromelain had a slightly higher glucose level than the normal control group and both groups combined ($p > 0.05$). Neither the normal control group nor the groups treated with Bromelain-derived bitter gourd seed protein hydrolysate demonstrated a statistically significant decrease in red blood cell (RBC) counts ($p \geq 0.05$). In this study, the white blood cell (WBC) count compared to the normal control group and groups treated with 100 and 200 mg/kg body weight (T100 and T200) of bromelain-derived bitter gourd seed protein hydrolysate showed a significantly elevated value ($p \leq 0.05$). The groups treated with 400 and 800 mg/kg body weight (T400 and T800 respectively) of this hydrolysate had significantly higher WBC counts. Additionally, the research found that hemoglobin (HBG) levels did not differ significantly between the groups treated with bitter gourd seed protein hydrolysate formed from bromelain and the normal control group, as well as between the treated groups themselves ($p \geq 0.05$). However, when compared to the normal control group and groups treated with 100 and 200 mg/kg body weight (T100 and T200) of bromelain-derived bitter gourd seed protein hydrolysate, respectively, there was a notable rise in packed cell volume (PCV) count in groups treated with doses of 400 and 800 mg/kg body weight (T400 and T800) of this hydrolysate ($p \leq 0.05$). When comparing the normal control group and the groups treated with bitter gourd seed protein hydrolysate generated from bromelain, there is no discernible alteration in the platelet (PLT) count in the rats ($p > 0.05$). According to 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

Toxicology studies on medicinal plants or their extracts are often used to establish the degree of safety, particularly in the development of novel drugs. The therapeutic effects of several chemicals and substances originating from plants are gaining more and more recognition. Traditional healers do not examine the potentially harmful effects of herbal medicines on certain organs in both people and animals. This has caused medical concerns. The fact that the active ingredient is just one of several components in the basic form that most herbal medicines are administered in raises concerns about organ toxicity (Mensah, 2019).

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When comparing the treated group to the normal control group and the hematological parameters (white blood cell count, red blood cell count, hemoglobin, packed cell volume, and platelet count), no significant changes were seen ($p > 0.05$).

groups. Bromelain hydrolysate from bitter gourd seeds may not be harmful to the hematopoietic system, meaning it won't cause any noticeable changes to the body's physiology. The hematopoietic system is a key indicator of health or illness and one of the most vulnerable organs to harmful substances.

At a greater dosage of 800 mg/kg body weight, the researchers found that the white blood cell (WBC) count increased significantly ($p < 0.05$). Infection, stress, inflammation, trauma, allergies, or specific diseases affecting an organ, tissue, or the whole body may manifest as high white blood cell (WBC) counts in numerous situations (Shcrier, 2010). The increased white blood cell (WBC) count in the treated rats may have been a result of toxic effects produced by the greater dosage of bitter gourd seed protein hydrolysate obtained from bromelain utilized in this investigation. This is due to the fact that WBCs are recognized as having a crucial function in the immune system of animals. They test the blood for infectious agents including viruses, bacteria, and fungus. White blood cells (WBCs) or leukocytes detect and remove the invading particle from the bloodstream before it causes sickness, usually caused by a virus or bacterium. Shcrier (2007) states that... Acute oral toxicity effects of Momordica charantia on Sprague-Dawley rats were studied by Husna et al. (2013) and Solomon and Akinbo (2022) on Histopathological and haematological changes observed in adult wistar rats administered with momordica charantia (bitter melons) aqueous leaf extract. However, this research contradicts those findings. This, however, is in line with what Umar (2017) found when he studied the effects of oral Momordica charantia treatment on hematological parameters in adult albino rats.

In this investigation, there was no significant change in the red blood cell (RBC) count between the control and treatment groups. Red blood cell (RBC) counts below the normal range are thought to be an indicator of anemia. Anemia may be caused by a lack of iron or certain vitamins, excessive blood loss, or a medical disease (Umar 2017).

The results of this investigation suggest that hydrolysate of bitter gourd seed protein generated from bromelain may be hepatotoxic at larger doses, since the values of alanine and aspartate aminotransferases (ALAT and ASAT, respectively) were seen to be raised.

Zhu et al. (2012) found that bitter melon powder's antidiabetic activity was affected by its superfine grinding, but after oral administration of a bitter gourd seed protein hydrolysate derived from bromelain, there were no significant changes ($p \geq 0.05$) in the levels of CHOL, HDL, LDL, and TG. The current investigation found no significant impact of bitter gourd seed protein hydrolysate generated from bromelain on the parameters of the lipid profile.

It seems that the bitter gourd seed protein hydrolysate and the bromelain-derived bitter gourd seed extract may not be nephrotoxic as they did not significantly alter blood urea and creatinine concentrations when taken orally. In clinical diagnostics, serum urea and creatinine are also used as indicators of renal functioning. The study was conducted by Madaki et al. in 2021.

5. Conclusion

Finally, at higher dosages, the hematological parameters (WBC, PCV, ALAT, and ASAT) have been shown to be negatively affected by the oral administration of bitter gourd seed protein hydrolysate generated from bromelain. An rise in levels of alanine and aspartate aminotransferases (ALAT and ASAT, respectively) is seen at a higher dosage of 800 mg/kg body weight of bitter gourd seed protein hydrolysate generated from bromelain. No changes were observed in red blood cell count, hemoglobin, or platelet count when bromelain-derived bitter gourd seed protein hydrolysate was taken orally. However, at 400 and 800 mg/kg body weight, there was a noticeable change in white blood cell count and packed cell volume, suggesting that this compound could be useful as a white blood cell and blood cell booster in infectious or anemic conditions or for prevention purposes. Although this study did not determine the exact mechanism(s) by which bitter gourd seed protein hydrolysate derived from bromelain increased white blood cell and packed cell volume, it is believed that this hydrolysate may directly affect the body's system that makes blood cells and may contain components that interact with and stimulate the production of lymphocytes and blood cells. It appears that the bitter gourd seed protein hydrolysate derived from bromelain may not be nephrotoxic, since it did not significantly alter the concentrations of serum lipid profile parameters (CHOL, HDL, LDL, and TG), urea, and creatinine when administered orally at varying doses. In clinical diagnostics, serum urea and creatinine are also used as indicators of renal functioning.

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