

Effect of processing on chemical composition, nutritional properties, and toxicity of *Mucuna pruriens* (L.) DC. Seeds

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World Journal of Advanced Research and Reviews, 2025, 27(02), 857–868

Publication history: Received on 17 July 2025; revised on 09 September 2025; accepted on 11 September 2025

Article DOI: <https://doi.org/10.30574/wjarr.2025.27.3.3143>

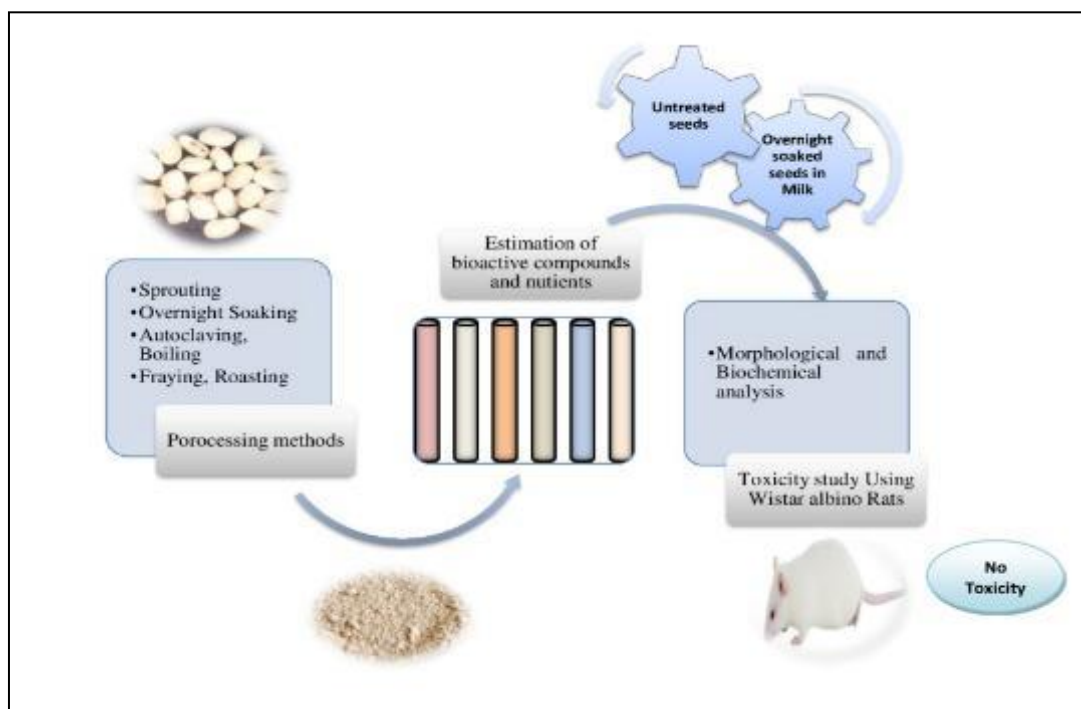
Abstract

Despite their high protein content, *Mucuna* is only a minor food crop, mainly because they contain anti-nutrients such as L-dopa. By analyzing the impact of several processing techniques on the protein quality and bioactive components of *Mucuna* beans, the possibility of using them as an alternative source of protein was determined. The amounts of phenolics, tannin, and L-Dopa were reduced by overnight soaking in milk to 83.04%, 61.92% and 63.92% respectively, while the amount of carbohydrates was raised by 14.71%. The same treatment resulted in the least (3.26%) reduction of protein content. Autoclaving reduced the phytate content up to 40.62% and the oxalate content was absolutely removed by sprouting, boiling with NaCl, overnight soaking in cow dung and urine. The flavonoid content in roasted seeds increased significantly by 339.52%. Unprocessed and overnight-soaked seeds in milk were used for the toxicity assessment using the Albino Wistar Rat model according to OECD 425 TG. The levels of S.G.P.T. (A.L.T.) and creatinine were significantly decreased in processed seed-treated rats, while no appreciable differences were seen in body weight, behavior, and haematological parameters in comparison to the control group.

Keywords: Protein; Food; Toxicity; Anti-nutrients; Processing; Bioactive compounds

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Graphical Abstract



1. Introduction

Mucuna is a genus of climbing vines and shrubs belonging to the Leguminosae family with more than 100 species found throughout the world in tropical and subtropical climates [1]. It is regarded as a reliable source of dietary proteins. It has high protein content (23–35%) and its digestibility is comparable to that of other pulses such as soybeans and lima beans [2]. Consumption of *Mucuna* beans increases serum level of L-DOPA, which increases the concentration of dopamine in peripheral tissues and causes ant physiological side effects such as nausea, vomiting, anorexia, paranoid delusions, hallucinations, delirium, severe depression, and dementia without outward signs of disease [3]. Phytic acid (phytate), tannins, cyanogenic glucoside (cyanide), oxalate, and gossypol are naturally occurring antinutritional components that limit the use of raw *Mucuna* beans as food for humans [4]. Antinutrient compounds alter the nutritional content of grain legumes and decrease meal intake and utilization of nutrients in animals, and their presence in food and feed is considered undesirable from a nutritional point of view [5]. However, a large number of research papers have recently examined the health benefits of these bioactive chemicals. In particular, these bioactive molecules have been shown to have a wide range of beneficial medicinal properties, including possible antioxidant activity [6]. A growing body of chemical, biochemical, clinical, and epidemiological evidence suggests a link between legume seed consumption and an overall reduction in the incidence of chronic diseases such as cancer, heart disease, diabetes, and obesity [7].

To enhance nutritional quality and fully utilize dried legumes as food, it is necessary to inactivate or remove antinutritional elements using commercially available processing methods. In addition to customer preferences, the chosen cooking method has a significant impact on the chemical content of the food, as well as the intake of bioactive components [8]. Legumes are consumed domestically after ordinary processing; some of the antinutritional components have been found to be reduced by simple processing techniques such as soaking and boiling [9,10]. However, it is understood that some processes, such as heat processing, could induce physicochemical changes in proteins, starch, and other components of legume seeds that would affect their nutritional characteristics [11]. It has been demonstrated that in boiling and roasting processes, heat is employed for the significant removal of antinutritional factors, including trypsin inhibitors, phytic acid, oligosaccharides, tannins, and L-DOPA [12], but this simultaneously affects nutritional factors, especially protein, carbohydrates, vitamins, minerals, and energy levels. Therefore, it is very important to find a reliable processing method that minimizes the loss of bioactive compounds and removes inhibitory compounds. The present study focuses on the use of traditional Indian seed processing techniques such as roasting, frying with ghee, boiling, and soaking in cow dung and urine to remove antinutritional factors and preserve the nutritional properties of seeds. Raw and processed *M. pruriens* L. seeds were also used for toxicity studies using the Albino Wistar Rat model.

2. Materials and Methods

2.1. Seed collection

The seeds of 57 *M. pruriens* accessions were collected from different geographical locations and screened for protein content. Seeds with the highest protein content were treated as follows:

2.2. Processing Methods

The dried *M. pruriens* seeds were sorted, cleaned, and in equal portions subjected to different treatments, such as roasting, frying, autoclaving, germination, overnight soaking in water, buttermilk, milk, coconut water, cow urine and dung, and boiling with NaCl. Raw seeds served as the control.

2.3. Determination of bioactive compounds

The Folin Ciocalteu Reagent Method [13] was used to determine the total phenolic content. The modified colorimetric approach reported by Abdel-Hameed [14] was utilized to calculate the flavonoid content. Oxalic acid was calculated using the method outlined by Mishra et al. [15]. The phytate content was determined using the titrimetric approach published by Abifarín et al. [16]. Tannins were estimated using the method described by Deutschla et al. [17]. L-DOPA was determined using the method adopted by Rani et al. [18]. The crude protein content was estimated by multiplying the percentage of Kjeldahl nitrogen by a factor of 6.25 [19]. Carbohydrates were assessed using the phenol-sulfuric acid method [20].

2.4. Toxicity Study

The acute oral toxicity study was conducted according to OECD guideline No. 425 (Up-and-down procedure). In the present study, healthy Albino Wistar rats (150–200 gm) between 8–12 weeks of age were used.

2.5. Acute toxicity assay

Rats were randomly selected, labelled to allow easy identification and housed in cages for five days prior to dosing. An acute toxicity investigation was conducted using a limit test at 2000 mg/kg per organism [21].

2.6. Haematological and Biochemical analysis

Blood samples were collected in EDTA-containing tubes from animals (treated and vehicle control groups) for a haematological study. Haemoglobin (Hb), total red blood cell (RBC), and platelet count were determined from the collected blood. Different biochemical parameters were measured on a biochemical analyzer using redox kits. The criteria used were alkaline phosphatase and alanine transaminase content for liver function and creatinine content for renal function.

2.7. Data Analysis

All results of this study were reported as means of three replications. Means and standard deviations for different physicochemical characteristics, nutrients, and bioactive compounds were calculated using SPSS software. A one-way analysis of variance (ANOVA) was performed to determine the significant differences between the means among the different treatments at $P < 0.05$.

3. Results and Discussion

In general, raw legumes are known to possess a much higher amount of antinutritional constituents than their processed counterparts, so processing is necessary before the legumes are added to food. Raw *Mucuna* seeds are a good source of bioactive compounds and nutrients [2]. Compared to raw seeds, the processing methods employed on the dried *M. pruriens* seeds significantly reduced ($p < 0.05$) the amount of phenolics, tannins, phytic acid, oxalate, and L-DOPA.

3.1. Effect of processing methods on bioactive compounds

Table 1 summarizes the bioactive compounds present in the raw and treated *Mucuna* seeds. Zeb et al. [22] observed that some phenolics, such as sinapine, catechin, and leucoanthocynidin, were significantly reduced by dry and moist heating (under pressure), which is supported by the fact that the amount of phenolics was reduced to 87.17% in autoclaved seeds compared to untreated seeds. Similar observations have also been reported in *Mucuna* [23,24,25].

Table 1 Effect of processing on bioactive compounds

Sr. no	Processing method	Phenolics (mg/100g)	Flavonoids (mg/100g)	Tannin (mg/100g)	Oxalate (mg/100g)	Phytic acid (mg/100g)	L-DOPA (mg/100g)
1.	Roasted	57.29±1.09***	7.34±0.25**	165.39±3.30*	0.04±0.01**	217.46±15.32*	0.27±0.47
2.	Fried	72.00±2.01**	7.22±0.03***	143.61±2.05**	0.28±0.11**	207.22±9.16*	0.24±0.07
3.	Autoclaved	19.61±0.98**	1.32±0.12	195.72±3.77*	0.29±0.11***	161.82±11.44**	0.21±0.12*
4.	Boiling in 1% NaCl for 1 hr	58.22±1.61**	1.30±0.03	155.40±2.38**	-	73.13±12.98*	0.15±0.12**
5.	Overnight Soaked	29.36±1.03***	1.59±0.066	101.10±2.83***	0.05±0.02**	219.69±13.99*	0.12±0.18***
6.	Sprouted	47.01±2.27***	1.72±0.04	158.26±4.05*	-	206.27±11.06*	0.14 ±0.07**
7.	Overnight soaked in Buttermilk	33.25±2.29	0.61±0.09*	98.66±1.50***	0.87±0.33*	183.50±7.81*	0.16±0.08**
8.	Overnight soaked in Milk	25.91±0.86**	3.26±0.05**	81.36±2.30***	0.31±0.12**	203.13±19.55*	0.11±0.04**
9.	Overnight soaked in coconut water	27.43±1.37**	2.06±0.05*	126.13±3.59**	0.17±0.06**	229.37±8.84*	0.14±0.08**
10.	Overnight soaked in Cow urine	42.51±1.75**	3.98±0.02***	167.139±3.46*	-	222.62±24.71*	0.16±0.10***
11.	Overnight soaked in Cow dung	36.72±1.54***	1.34±0.10	153.48±3.38*	-	211.55±15.43*	0.23±0.09
12.	Raw seeds	152.82±3.91	1.67±0.02	213.68±3.60	2.13±0.84	272.55±17.96	0.29±0.05

n=3. Data are expressed as mean ± SD: *Represents significant correlation at $P < 0.05$

Other treatments, such as overnight soaking in milk, coconut water, and water, are comparable to autoclaving, reducing the total phenol concentration by more than 80%. On the other hand, overnight soaking in buttermilk, cow urine, and cow dung reduced total phenols by more than 70% [26]. A significant reduction of 80% of phenols in *Mucuna* seeds has been achieved after soaking treatment followed by irradiation by Siddhuraju et al. [27]. Sprouting, cooking with 1% NaCl and open-pan roasting resulted in a loss of more than 60% of the phenolic compounds [26], while frying [52.89%] resulted in the least reduction.

Numerous seeds and grains have also been observed to contain more total flavonoids after being subjected to various roasting methods [28,29]. The flavonoid content of roasted and fried seeds increased by 339.53% compared to raw seeds. Overnight soaking in milk and cow urine also increased the flavonoid concentration to 100%, followed by overnight soaking in coconut water (23.50%). Boiling with 1% NaCl and autoclaving significantly reduced the number of flavonoids by up to 20%, supporting the findings of Siah et al. [30] that the total flavonoid content was adversely affected by boiling rather than autoclaving in cowpea. Because flavonoids are polyphenols and all polyphenols are water-soluble, the flavonoid content is reduced during boiling [31]. The least reduction was observed after the overnight soaking in water (4.79%).

Tannins are heat stable and decrease protein digestibility in animals and humans, probably due to partially unavailable protein or inhibiting digestive enzymes and increasing fecal nitrogen [32]. The greatest tannin loss was observed in seeds soaked overnight in milk, up to 61.92%, followed by seeds soaked overnight in buttermilk, water and coconut water due to the leaching of tannins into the soaking medium [33,34]. Tannins were eliminated from grains during germination due to polyphenolase-induced enzymatic breakdown [35]. The loss of tannin content was 35% to 20% in the other treatments. Fitriani et al. [36] reported similar results in Kabau, where soaking, boiling, and pressure cooking resulted in a significant decrease in tannin content.

Oxalates prevent calcium absorption by forming insoluble salts with calcium. Boiling in 1% NaCl for 1h, sprouting, overnight soaking in cow urine and dung completely removed the oxalate content from the seeds. A drastic reduction in oxalate content could be due to the loss of leaching from soaking and boiling [37]. Roasting, overnight soaking in water, and coconut water removed up to 98% of oxalate. Similar findings have been observed in yellow maize [38] and *Mucuna* seeds [39] soaked for 12 hours. Frying and autoclaving reduced the amount of oxalate in Bambara groundnut by up to 86%, supporting the findings of Adeleke et al. [40]. Elimination of 80% of the oxalate content was also achieved by overnight soaking in milk, while the least reduction of 59.15% was observed in buttermilk-soaked seeds compared to the control.

The availability of minerals in animals' digestive tracts is increased when phytic acid level is reduced because the chelating capacity of phytic acid is reduced [41]. Autoclaving was found to be the most effective method of reducing phytic acid levels by 40.62%. According to previous reports, it can reduce the phytate content up to 65.04–70.49% [33,42,43,44]. Boiling with 1% NaCl resulted in a 36.47% reduction in phytic acid, indicating that boiling is more effective than roasting [45]. The heat lability of phytic acid and the development of insoluble complexes between phytate and other components may contribute to the reduction of phytic acid content after heat treatment [46]. Overnight soak in buttermilk, milk, and sprouts resulted in a 25%–30% reduction. Similar findings have also been reported for African yam and Red kidney beans [47]. Overnight soak in water, cow dung, and urine and roasting resulted in a loss of 18–28% of phytic acid [39,44]. Frying beans with ghee reduced the phytic acid content by 23.96% compared to the control. The phytic acid content was lowest in seeds soaked overnight in coconut water treatment (15.84%), as a 12-hr soaking period may be inadequate to reduce the phytic acid content.

Although L-DOPA is an active ingredient in the pharmacological sense, it is potentially antinutritional and toxic if ingested in large amounts [34]. Overnight soak in milk eliminated 62.06% of the L-DOPA content, followed by overnight soak in water (58.62%) and coconut water (51.72%) treatments [41,43]. The sprouting resulted in a similar loss of L-DOPA (51.72%) [46,49]. Boiling in 1% NaCl for 1 hr and soaking in cow's urine resulted in 41–45% loss, whereas autoclaved seeds resulted in a reduction of 27.58%. This may be due to the leaching of L-DOPA from the seeds [34,44]. Overnight soak in cow dung treatment resulted in a 20.68% reduction in L-DOAP content, while roasting and frying reduced L-DOAP content by 17.24% and 6.89%, respectively [39, 48].

3.2. Effect of processing on protein and carbohydrate content

Mugendi et al. [39] studied the effect of processing methods on the nutritional composition and antinutrient content of *Mucuna* beans and found that the processing method used reduced the antinutritional constituents of *Mucuna* beans but ultimately resulted in loss of protein. The protein content of raw *Mucuna* seeds was approximately 33%, (Table 2) which is comparable to that of Soybean (35–40%) [49], Chickpea (18–22%) [50] and Cowpea (23–22%) [51]. The

protein content in sprouted seeds increased to 4.37% [52]. This is mainly due to the substantial breakdown of seed storage substances and the production of structural proteins and other cell components during germination [39]. The lowest reduction observed was 3.26% in seeds soaked overnight in milk. Overnight soaking in water, coconut water, cow dung, and urine resulted in a 25% to 35% protein loss, while a maximum reduction was observed after overnight soaking in buttermilk treatment (62.30%). The decrease in protein content can be attributed to the leaching of low-molecular-weight nitrogen compounds [53]. Boiling in 1% NaCl for 1hr removed 30.66% of the protein content, while autoclaving resulted in up to 40.14% loss. The decrease in protein content in the hydrothermal process could be due to the efflux of soluble protein into water [54]. Protein leaching occurs, particularly at high temperature and pH. Repeated soaking in tap water at room temperature also resulted in a 50% loss of protein from *Mucuna* bean flour [55]. Similarly, in the roasting process, the protein content decreased marginally as the denaturation of heat-sensitive nitrogenous compounds from the seeds occurred, resulting in a protein loss of 54% [25,56]. The decrease in protein content during frying (74.99%) could be due to the processing temperature. High temperature could induce new molecules' formation as amino acids. These amino acids can react with other compounds, such as aldehydes, epoxides, and hydroxyl ketones. The Maillard reaction also occurs during the frying process [36].

Table 2 Effect of processing on protein and carbohydrate content

Sr. no	Processing method	Carbohydrate (%)	Protein (%)
1	Roasted	37.31±0.32	15.18±1.16**
2	Fried	42.18±0.78*	8.26±0.60***
3	Autoclaved	42.99±0.68**	19.77±0.80**
4	Boiling in 1% NaCl for 1 hr	32.48±1.14*	22.90±0.66**
5	Overnight soaked	31.61±0.61*	21.06±1.07**
6	Overnight soaked in buttermilk	37.59±0.56	12.45±0.97**
7	Sprouted	34.55±1.26	34.54±0.32
8	Overnight soaked in milk	42.34±1.35*	31.95±0.55**
9	Overnight soaked in coconut water	29.49±0.72*	20.82±0.95**
10	Overnight soaked in cow urine	32.48±1.48*	23.65±0.46**
11	Overnight soaked in cow dung	24.51±1.13*	19.59±0.34***
12	Raw seeds	36.91±0.63	33.03±0.07

n=3. Data are expressed as the means: *Represents significant correlation at $P < 0.05$

Autoclaving increased the amount of carbohydrates to 16.47%, followed by overnight soaking in milk (14.71%) [57]. Autoclaving also increased the carbohydrate content of faba beans [58]. Fried seeds also showed an increase in carbohydrates, up to 11.56%, followed by an overnight soak in buttermilk (1.84%). On the other hand, overnight soaks in cow dung (64.5%), coconut water (20.10%), and water (14.35%) resulted in the greatest loss of carbohydrates. Overnight soaking in cow urine and boiling in 1% NaCl for 1hr resulted in a similar (12.00%) reduction, while sprouting (6.39%) and roasting (1.08%) resulted in the least reduction compared to raw seeds. Similar observations have been reported on the reduction of dietary carbohydrates after heating, autoclaving, and roasting of *Mucuna* seeds [59,25].

Overnight soaking in milk and sprouting treatment resulted in a greater loss of bioactive compounds while preserving protein and carbohydrate content. As sprouting in *Mucuna* beans requires 72 hours [39,42], overnight soaking with milk is preferred for the assessment of toxicity in Albino Wistar rats.

3.3. Toxicity study

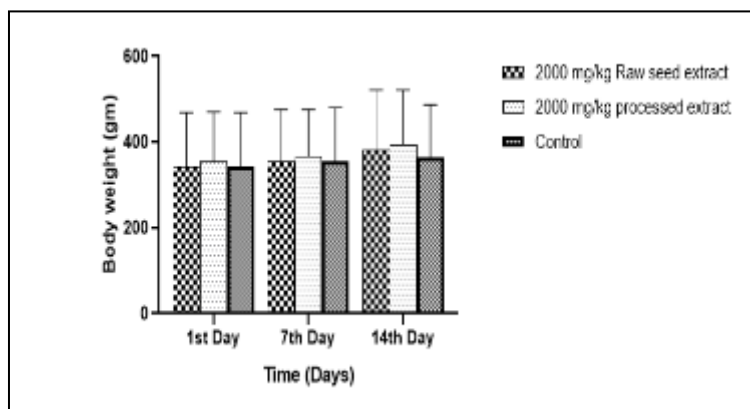
A limit test was conducted with a dose of 2000 mg/kg body weight of *M. pruriens* seed extracts. Test animals were observed for the first 30 min after the administration of the extracts, followed by 3hr, 28hr and 48hr intervals, and various signs and symptoms related to the mental, physiological and autonomic profiles of the animals were continuously monitored. No noticeable changes were observed in the wellness metrics used to assess toxicity (Table 3), and no mortality was observed during the study period. Therefore, the LD50 should be more than 2000 mg/kg of body weight. These results are consistent with earlier studies by Hadimani et al. [60] and Saikarthik et al. [61].

Table 3 Rat behavioural patterns in groups treated with plant extracts at 2000 mg/kg

Sr. No	Parameters	30minutes			3hr			24hr			48hr			7days			14 days		
1		C	R	T	C	R	T	C	R	T	C	R	T	C	R	T	C	R	T
2	Fur & skin	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	Eyes	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	Respiration	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	Urination (colour)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	Faeces consistency	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8	Somatomotor activity & behaviour pattern	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9	Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	Convulsions & tremors	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
12	Itching	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
13	Coma	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
14	Mortality	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

C- Control; R- Raw seeds; T, treated seeds; A- Absent; N- Normal

One of the human end objectives is fluctuations in body weight, which are believed to be a sensitive sign of an animal's general health [62]. Figure 1 shows the body weights of all animals, both control animals and treated animals. No significant changes in body weight were observed after treatment with the different extracts. The brain, liver, kidneys, and heart are the main vital organs affected by toxic substances [63].

**Figure 1** Effects of seed extracts on the body weight of Albino Wistar rats in the acute toxicity study.

The basic data necessary to determine whether the weight of an organ is susceptible to damage is its relative weight. The heart, lungs, kidneys, spleen, and stomach were observed after sacrifice (Figure 2). No lesions or damage were found on histological examination. The liver is the target site for most toxic metabolism, which can lead to damage to the membrane permeability of this organ and the leakage of liver enzymes into the blood stream [64]. Therefore, the quantification of liver marker enzymes in the animal system is one of the most common approaches adopted to evaluate the toxicity of a plant extract [65]. Alanine aminotransferase and creatinine levels were significantly decreased in processed seed-treated rats, while alkaline phosphatase, total protein, albumin, globulin, and the A/G ratio showed no significant changes compared to the control (Figure 3). Maxwell & Yusuf [66] also observed that the application of processing methods decreases Alanine aminotransferase levels and lowers liver toxicity in albino rats. Increased

creatinine level is a significant indicator of damaged functional nephrons [67], indicating that the processing method reduces the toxic effects of antinutrients on the kidneys. Haematological parameters are sensitive indicators of physiological alterations in response to any type of toxic stress or environmental pollutants [68]. Statistically, no appreciable variation in total blood count suggests that the seed extract had no influence on haematological markers (Figure 4).

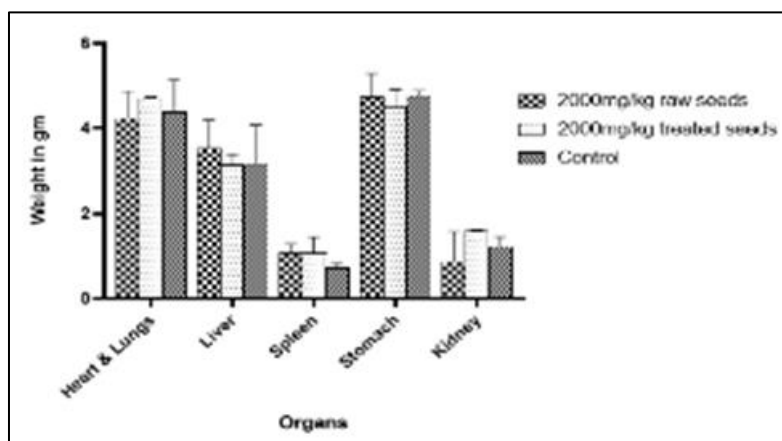


Figure 2 Relative organ weights of rats after receiving the extract of *M. pruriens* seeds for 14 days

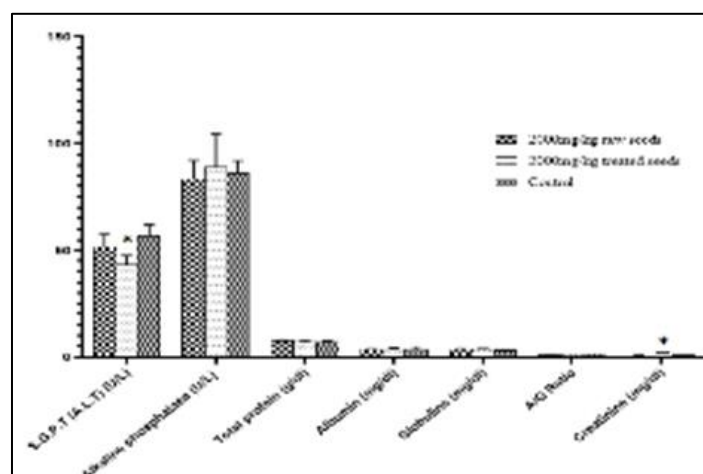


Figure 3 Effect of seed extracts on liver and kidney function tests of Albino Wistar rats

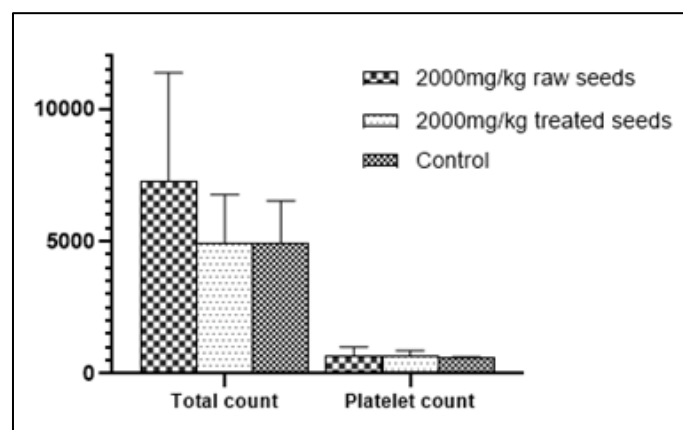


Figure 4 Effect of seed extracts on CBC

4. Conclusion

The processing efficiency of the method was defined based on the two most important parameters, protein content and residual L-DOPA, which determine the quality of the processed *Mucuna* beans. Other bioactive compounds, such as phytates, tannins, oxalates, and phenolic compounds, were also significantly reduced. Some reduction in crude protein (approximately 3%) was observed during processing. However, the carbohydrate content of processed *Mucuna* beans was higher than that of commonly consumed legumes. With regard to protein quality, sprouting and overnight soaking in milk would be the best methods. However, the processed beans given as the sole protein did not support the growth of Albino Wistar rats. The lethality of the seed was further checked on the functional parameters of the liver and kidney, and significant reductions in Alanine aminotransferase and creatinine levels were observed. Therefore, overnight soaking of seeds in milk reduces the effort and processing time compared to sprouting and preserves the nutritional value of food, thus ensuring high-quality nutrition to maintain human health. The information gleaned from this study could be used to improve food security, as processed *Mucuna* beans have great potential as a low-cost alternative protein source.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared no conflict of interest regarding the publication of this paper.

Statement of ethical approval

The animal study was approved by the Ethical Committee of Uka Tarsadia University (MPC/IAEC/02/2021).

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