

ORIGINAL ARTICLE

Effect of *Stephania abyssinica* Leaves Powder Supplementation on some Biochemical Parameters and Immune-Nutritional Status of Malnourish early-Weaned Rats

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Abstract

Child malnutrition is a severe public health problem in Africa as a whole and Cameroon in particular. The objective of this study was to assess the effectiveness of *Stephania abyssinica* leaf powder as a nutritional supplement to treat protein-energy malnutrition in immature rats. The proximate composition of *S. abyssinica* leaves was assayed using standard methods. The 21 days old weaned rats were fed with low protein diet (2.5%) during 28 consecutive days. They were further fed with either normal diet (20% protein) or 2.5% protein diet supplemented each with *S. abyssinica* leaves powder at different doses (110 mg/kg, 220 mg/kg and 440 mg/kg) for 28 days. The establishment of protein-energy malnutrition and the recovery of rats following treatment were evaluated by determining, hematological parameter and transaminases activity.

The proximate composition of *S. abyssinica* leaves showed that they are made up of 95.30% dry matter. Dry matter analysis revealed that it contains 27.54% crude proteins, 4.41% lipids, 54.12% carbohydrates and 9.23% ash content. *S. abyssinica* leaves as food supplement significantly improved the animal's health and immune system.

Practical application

S. abyssinica could be useful as food supplement to fight and prevent infant malnutrition as the powder displayed no toxicity on liver.

Keywords: Protein-energy malnutrition, immune system, Immune nutritional status, *Stephania abyssinica*.

1. Introduction

Protein energy malnutrition (PEM) is a range of pathological conditions arising from inadequate proportions of proteins and calories occurring most frequently in infants and young children and commonly associated with infections (Muscaritoli *et al.*, 2009). It is the most frequent type of malnutrition, affecting at least 800 million people worldwide (De Onis *et al.*, 1993). Children below 5-years old, especially those in developing countries, present a deficiency of more than two standard deviations in relation to the WHO/NCHS body weight standards for their

age (193 million), to height standards (230 million) and weight to height ratio standards (50 million) (Tomkins, 2000). Severe protein energy malnutrition in early life can permanently change the growth, body form, structures and functions (Obimba, 2012). However, the long term effect of malnutrition depends on the stage and intensity of its occurrence (Barker, 1994). The alteration can include linear growth retardation and a significant reduction in body weight, reduced cell number in tissues and organs leading to the modification of organ structure,

selection of particular clones of cells and change in metabolic differentiation (Waterland & Garza, 1999; Camargo & Almeida, 2005). Poor nutrition impairs various functions of the immune system causing alterations in different components of both specific and non-specific immunity (Fukushima, 2004). It is also the main cause of immune depression, modifying both the adaptive and innate immune system, as well as impairing hematopoiesis by altering T-dependent areas of the lymphoid tissue, decreasing phagocytosis, hindering respiratory burst, reducing nitric oxide availability, down-regulation pro-inflammatory cytokine production (Borelli *et al.*, 2009). Protein malnutrition has long been recognized as a common problem. Ignorance and poverty are key factors in the etiology. So, shortage of animal protein for human consumption and the prevalence of protein malnutrition in developing countries probably need supplementation in the diet with protein from plant sources (Bayoumy, 2013). Hence, it has become necessary to look into local diets, especially those that are sources of good quality protein with a view of ascending their efficacy in the prevention and/or rehabilitation of cases of protein energy malnutrition (Runsewe-Abiodun *et al.*, 2001).

S. abyssinica is a plant commonly used in the West region of Cameroon to treat children affected by malnutrition. The present work was carried out in order to assess the effectiveness of *Stephania abyssinica* leaf powder as a nutritional supplement to treat protein malnutrition in immature rats.

2. Materials and Methods

2.1. Materials

2.1.1. Plant material

S. abyssinica leaves were used throughout the experiment. The plant was collected in Bamenyan village, Bamboutos division, West region, Cameroon. The identification was carried out at the Cameroon National Herbarium in Yaoundé where a voucher specimen was deposited under the registration number 17046/H.N.C.

2.1.2. Animals

Wistar albinos rats of 21 days old weighing 25 to 44 g were used for the experiment. These animals were bred in the animal house of the University of Dschang and lodged in plastic cages under normal laboratory conditions (12 hours light/dark cycle: 23±2°C). They were fed with standard rat diet (Telefo, 1998). Food and water were given ad libitum to all animals throughout the experiment period. These animals were handled according to standard protocols for the use of laboratory animals. The studies were conducted according to the ethical guidelines of the Committee for Control and Supervision of Experiments on Animals (Registration no. 173/CPCSEA, dated 28 January, 2000), Government of India, on the use of animals for scientific research.

2.2. Methods

2.2.1. Proximate composition of *S. abyssinica* leaves

The moisture and ash contents were determined according to the AOAC methodologies (AOAC, 1990). The fat content was determined in the dry matter by using the Soxhlet extraction method, with chloroform as solvent (AOAC, 1990). This content was expressed in g of fat per 100 g of dry matter. The amount of protein present in dry leaves powder was determined by the analysis of the total nitrogen protein using the Kjeldahl method and expressed in g per 100 g dry matter

(DW) (Kjeldahl, 1883; Marizvikuru & Gwaze, 2013). Total carbohydrates content was determined as difference between 100 and the sum of water, protein, total lipid and ash content (AOAC, 1990). All measurements were performed in triplicate.

2.2.2. Protein malnutrition induction

Weaned animals were randomly distributed into two different groups of 20 and 60 animals. The first (A) group was fed with normal rat diet (20% proteins) and the second group (B) with a low-protein diet (2.5% proteins). Both diets were given for 28 consecutive days (Fontenla de Petrino *et al.*, 2007). The composition of the two diets is given in Table 1.

Table 1: Composition of the R0 and R1 diets (g/100 g)

Ingredients	Normal rat diet (R ₀) (20% proteins)	Hypoproteic diet (R ₁) (2.5 % proteins)
Soya bean flour	20.0	2.5
Corn flour	70.7	88.2
Minerals mixture	4.0	4.0
Maize oil	4.0	4.0
Vitamins	1.0	1.0
NaCl	0.3	0.3

Throughout the protein malnutrition induction period, food intake and weight gain was registered daily. At the end of 28th day, five rats in each group were anesthetized with chloroform vapor and blood samples collected by cardiac puncture into heparinized and non-heparinized tubes. The non heparinized tubes were allowed to clot for 8 hours and centrifuged at 3000 rpm for 10 min to obtain the serum. Animals were further sacrificed and used for gross pathological examinations. Blood collected in heparinized

tubes were used to assay the total red blood cells and white blood cells count using the Malassez chamber. Different sub-populations of white blood cells including lymphocytes, monocytes, basophils, eosinophils, and neutrophils were estimated by microscopic counting after May-Grunewald-Giemsa staining.

The albumin concentration was evaluated in the serum using commercial kit (Pointe Scientific, 5449 Research Dr, Canton, MI 48188, United State). Total protein concentration was also assayed in the serum using Biuret method (Gornall *et al.*, 1949).

2.2.3. Assessment of the correction of malnutrition

In order to evaluate the effect of the food supplemented with *S. abyssinica* leaves powder, animals fed with normal rats diet (A) were divided into two groups.

- A1(control): R0
- A2: R0 + *S. abyssinica* leaves powder (220 mg/kg bw)

Malnourished rats (B) were also separated into five groups.

- B0: R0 + *S. abyssinica* leaves powder (0 mg/kg bw)
- B1: R1 + *S. abyssinica* leaves powder (0 mg/kg bw)
- B2: R1 + *S. abyssinica* leaves powder (110 mg/kg bw)
- B3: R1 + *S. abyssinica* leaves powder (220 mg/kg bw)
- B4: R1 + *S. abyssinica* leaves powder (440 mg/kg bw)

Each subgroup was made up of five rats and the experiment was conducted during 28 consecutive days (Sall *et al.*, 1999).

Throughout the treatment period, the food intake and weight gain were recorded daily. On the 29th day of treatment, animals of each group were anesthetized, blood and serum were collected for haematological analysis. Albumin and total protein concentrations were also determined as mentioned above. In addition, the activity of transaminases (ALT and AST) was measured to evaluate the possible toxicity of *S. abyssinica* on rat liver. This was done using Pointe Scientific kits.

2.2.3.1. Peritoneal macrophages mobilization

The rats in each group were injected intraperitoneally 3 days before the end of the *S. abyssinica* powder supplementation with 2 mL of ovalbumin solution (30 µg/mL). On the 29th day, prior to blood collection, the peritoneal membrane was washed twice with 4 mL phosphate buffer saline, Ph 7. The peritoneal exudates were collected and centrifuged at 1000 rpm, 25°C for 30 minutes. The erythrocytes were further eliminated by hypotonic lysis and the cell pellet suspended in phosphate buffer saline. Total macrophage was assayed using Malassez chamber.

2.2.3.2. Delayed type hypersensitivity reactions

Rats in each subgroup were primed on day 7 following treatment with 50 µl of sheep red blood cells (SRBC) (5×10^6 cells/mL/animal) in Freund complete adjuvant by subcutaneous injection into the right hind footpad and challenged on day 14 by 50 µl of SRBC in Freund incomplete adjuvant. The contralateral foot pad received an equal volume of phosphate buffer saline in Freund's adjuvant. The thickness of each foot pad was measured 24 hours after the challenge with Freund complete adjuvant using a Vernier caliper. The difference in thickness of the right hind pad and the left hind pad was used

as a measure of delayed type hypersensitivity (DTH) reaction (Ghule *et al.*, 2006; Kanjwani *et al.*, 2008).

2.2.4. Statistical analysis

Data were submitted to the one way analysis of variance (ANOVA) and recorded as mean standard \pm SD and means were compared using Waller Duncan test at 0.05 significant level.

3. Results

3.1. Proximate composition of *S. abyssinica* leaves

The *S. abyssinica* dry leaves are made up of 95.30% dry matter. Analysis of the dry matter showed that it contains 27.54% crude proteins, 4.41% lipids, 54.12% carbohydrates and 9.23% ash.

3.2. Characteristics of protein malnutrition

The administration of low protein diet resulted in fur loss, change in color, and death (5%). After 28 days of low protein diet administration, rats showed low food intake compared to the control (data not shown). This resulted in a reduction in body weight (Figure 1) and physical activity. The induction of protein-energy malnutrition revealed a significant decrease in albumin and serum protein concentrations as well as total red blood cells, white blood cells, eosinophils, neutrophils, lymphocytes, monocytes and pack cell volume (Table 2).

3.2. Weight gain of malnourished rats treated with *S. abyssinica* leaves as food supplement

S. abyssinica leaves powder treatment administered as food supplement, independently to the diet, significantly increased the body weight of rats of all subgroups. This was more



Figure 1: Effects of protein-energy malnutrition on weight gain

S. abyssinica dry leaves powder, administered as food supplement independently of the diet significantly increased the albumin level of malnourished rat (Figure 3).

The total serum protein remained high when the malnutrition treatment was done with normal rat diet and *S. abyssinica* dry leaves powder. On the other side the supplementation of the low protein diet with *S. abyssinica* powder seemed to significantly reduce the level of total protein in the serum (Figure 4).

Table 2: Effect of protein energy malnutrition over a 28 days period on various blood parameters

	Malnourish	Control
Albumin (g/dl)	2.00 ± 0.36	3.90± 0.25
Serum Proteins (g/dl)	3.13 ± 0.90*	5.30 ± 0.78
Red blood cells (x10 ⁶ /mm ³)	3.56 ± 1.95*	6.31 ± 0.55
White blood cells (x10 ³ /mm ³)	4.82 ± 0.71*	43.40 ± 3.07
Neutrophils (%)	31.00 ± 6.87*	1.33 ± 0.36
Eosinophils (%)	1.20 ± 0.28*	1.00 ± 1.00
Basophiles (%)	0.60± 0.54	55.00 ± 7.54
Lymphocytes (%)	48.80 ± 8.11*	4.00 ± 1.18
Monocytes (%)	1.33 ± 0.57 *	43.5 ± 5.80
Packed cells volume (%)	31.33 ± 7.50*	

Values are expressed as Mean ± S.E.M, n = 5. * indicates the significant difference between the control group and the malnourished group P≤ 0.05%

visible when supplement was associated with a normal rat diet (Figure 2).

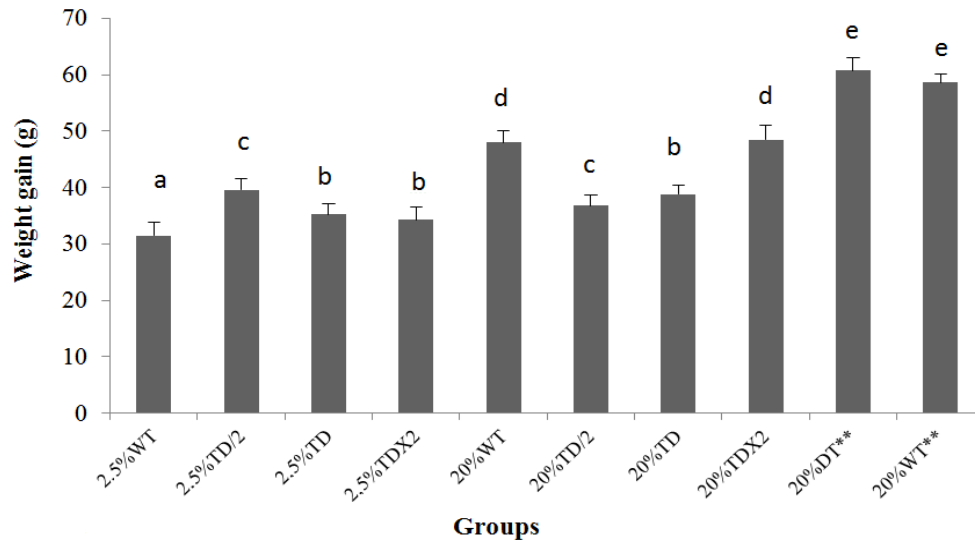
3.3. Serum albumin and serum protein concentrations of malnourish rats treated with *S. abyssinica* leaves as food supplement

3.4. Haematological parameters and immune response of malnourished rats treated with *S. abyssinica* leaves as food supplement

When given as food supplement to malnourished animals, *S. abyssinica* leaves significantly increased the level of red blood cells, white

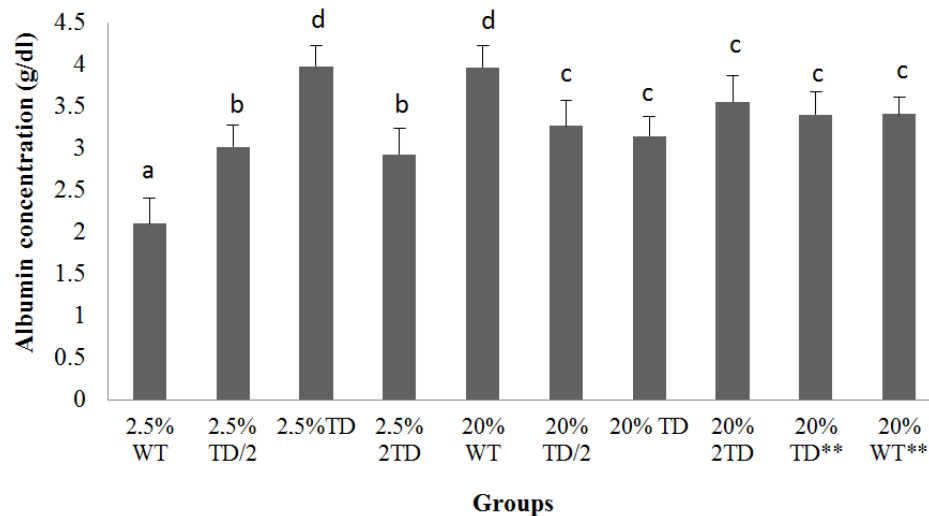
blood cells and packed cell volume. But when given at the highest dose together with normal rat diet (i.e. 440 mg/kg b.w) these parameters were significantly reduced (Table 3).

Malnourished animals under low protein diet (R1) without *S. abyssinica* leaves showed higher DTH response than those receiving the plant as supplement (Figure 5). In the same way, when



** two months animals that did not undergo malnutrition
 WT= without treatment, TD (therapeutic dose) = 220 mg/kg; TD/2= 110 mg/kg; TDx2= 440 mg/kg; Data are expressed as Mean ± S.E.M. n = 5. Values for a given group followed by same letter as superscript are not significantly different according to Waller Duncan multiple comparison procedure (at P < 0.05).

Figure 2: Effect of different doses of *S. abyssinica* leaves powder supplement on gain weight



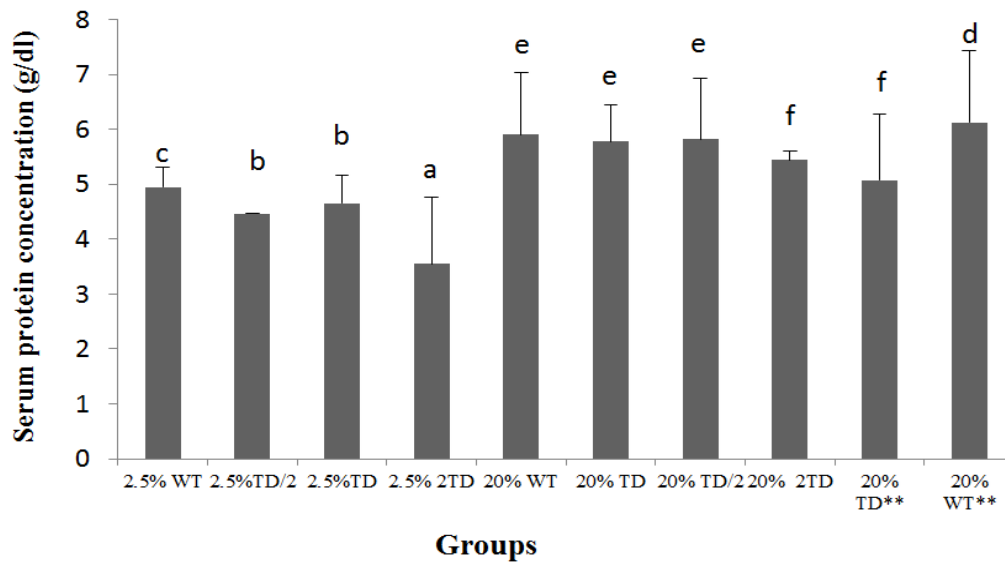
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Figure 3: Effect of different doses of *S. abyssinica* supplement on serum albumin concentration

Table 3: Effect of *S. abyssinica* dry leaves powder supplement on haematological parameters

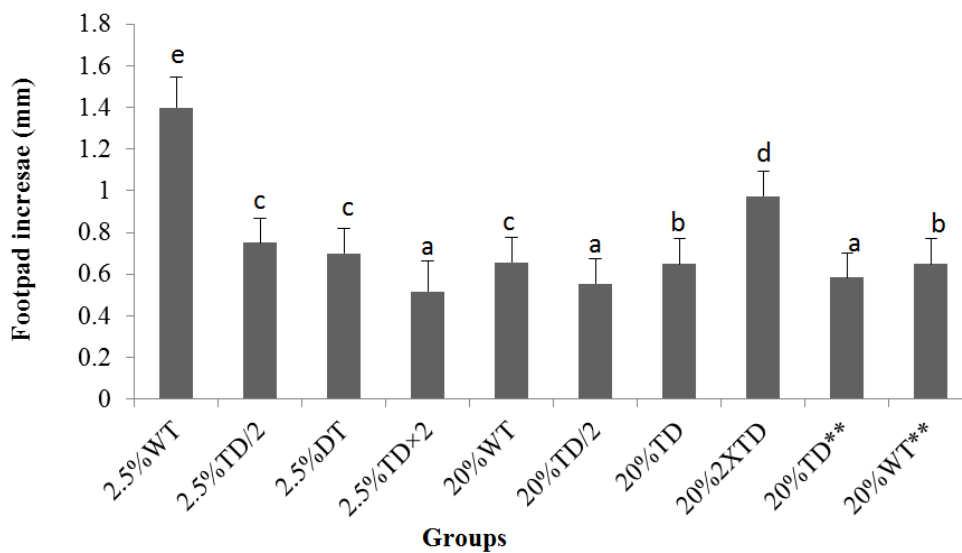
Groups	Hematocrit (%)	WBCs (x10 ³ /mm ³)	RBCs (x10 ⁶ /mm ³)	Neutrophils (%)	Eosinophils (%)	Basophils (%)	Lymphocytes (%)	Monocytes (%)
20% TD**	50.33 ± 3.38 ^d	6.75 ± 0.71 ^{c.d.e}	3.78 ± 0.19 ^b	31.33 ± 3.96 ^a	1.00 ± 0.36 ^a	0.66 ± 0.37 ^a	59.00 ± 3.89 ^d	8.00 ± 1.52 ^d
20% WT**	50.75 ± 2.38 ^d	6.90 ± 0.43 ^{c.d.e}	3.94 ± 0.15 ^b	43.43 ± 2.59 ^{a.b}	0.62 ± 0.22 ^a	0.50 ± 0.23 ^a	49.87 ± 2.38 ^{b.c.d}	5.50 ± 0.93 ^{c.d}
2.5% WT	21.56 ± 3.38 ^a	3.34 ± 0.71 ^a	2.62 ± 0.19 ^a	34.17 ± 3.72 ^{a.b}	0.33 ± 0.28 ^a	0.67 ± .37 ^a	36.00 ± 3.89 ^a	0.667 ± 0.570 ^a
2.5% TD/2	45.00 ± 2.62 ^{c.d}	7.68 ± 0.53 ^e	3.57 ± 0.15 ^b	40.50 ± 4.85 ^{a.b}	0.60 ± 0.28 ^a	1.00 ± 0.290 ^a	57.20 ± 3.01 ^{c.d}	3.80 ± 1.18 ^{a.b.c}
2.5% TD	46.67 ± 2.39 ^d	7.48 ± 0.50 ^e	3.90 ± 0.13 ^b	48.50 ± 4.85 ^b	0.83 ± 0.34 ^a	1.00 ± 0.26 ^a	47.17 ± 2.46 ^{b.c}	4.83 ± 1.07 ^{a.b.c}
2.5% 2×TD	47.53 ± 3.83 ^d	5.16 ± 0.71 ^{b.c}	3.73 ± 0.19 ^b	42.00 ± 6.87 ^{a.b}	0.66 ± 0.36 ^a	0.33 ± 0.37 ^a	51.00 ± 3.89 ^{b.c.d}	2.33 ± 1.52 ^{a.b}
20% WT	48.80 ± 2.61 ^d	5.60 ± 0.55 ^{b.c.d}	3.91 ± 0.15 ^b	45.50 ± 3.43 ^{a.b}	0.80 ± 0.28 ^a	0.80 ± 0.29 ^a	52.40 ± 3.01 ^c	1.60 ± 1.18 ^{a.b}
20% TD/2	45.77 ± 3.38 ^{c.d}	7.06 ± 0.55 ^{b.c.d.c}	3.95 ± 0.15 ^b	42.00 ± 4.85 ^{a.b}	1.00 ± 0.28 ^a	1.40 ± 0.29 ^a	53.60 ± 3.02 ^{b.c.d}	3.00 ± 1.18 ^{a.b}
20% TD	48.14 ± 2.21 ^d	6.41 ± 0.46 ^{b.c.d.e}	4.05 ± 0.12 ^c	44.33 ± 3.96 ^{a.b}	0.57 ± 0.23 ^a	0.86 ± 0.24 ^a	53.28 ± 2.50 ^{b.c.d}	4.71 ± 0.97 ^{a.b.c}
20% 2×TD	37.67 ± 3.38 ^{b.c}	5.69 ± 0.54 ^{b.c.d}	3.66 ± 0.16 ^b	44.00 ± 4.85 ^{a.b}	0.50 ± 0.33 ^a	0.75 ± 0.38 ^a	45.75 ± 3.37 ^{a.b}	4.50 ± 1.32 ^{a.b.c}

** two months animals that did not undergone malnutrition; WT= without treatment, TD (therapeutic dose) = 220 mg/kg; TD/2= 110 mg/kg; TD×2= 440 mg/kg; Data are expressed as Mean ± S.E.M. n = 5. Values for a given group followed by same letter as superscript are not significantly different according to Waller Duncan multiple comparison procedure (at P < 0.05).



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Figure 4: Effect of different doses of *S. abyssinica* supplement on serum albumin concentration



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Figure 5: Effect of different doses of *S. abyssinica* leaves powder supplement on delayed type hypersensitivity reactions (DTH)

this powder was given to rat receiving the normal diet, the delay-type hypersensitivity reaction was found to increase (Figure 5).

The *S. abyssinica* leaves powder significantly increased the number of macrophages at the peritoneal cavity when administered at 110 mg/kg b.w either supplemented with diet R0 or R1 (Figure 6).

3.5. Assessment of hepatic function following supplementation with *S. abyssinica* leaves

Administration of *S. abyssinica* leaves at doses more than 110 mg/kg bw. reduce the activity of serum activity of alanine transferase (ALAT) in both normal and malnourished animals groups. The decrease in AST activity was not significant with the supplemented of *S. abyssinica* leaves (Figure 7).

4. Discussion

Many plants are known in traditional medicine and used for treatment and management of malnutrition. The medicinal properties of these plants have been attributed to the biochemical composition of the plant materials (Fasuyi *et al.*, 2007). The proximate composition of *S. abyssinica* leaves revealed that they have an appreciable amount of nutrients. Moringa oleifera leaves are well regarded for their high concentration of nutrients, particularly protein. Oduro *et al.* (2008) revealed that these leaves are very nutritious when compared to vegetables such as cassava leaves, amaranth, mushrooms, taro leaves and pumpkin leaves. The present finding indicates that *S. abyssinica* leaves are made up of 27.54% crude protein. They are therefore richer in their protein than *M. oleifera* (16.15%). *S. abyssinica* leaves could therefore significantly contribute to the nutritional requirements of humans and should be strongly recommended.

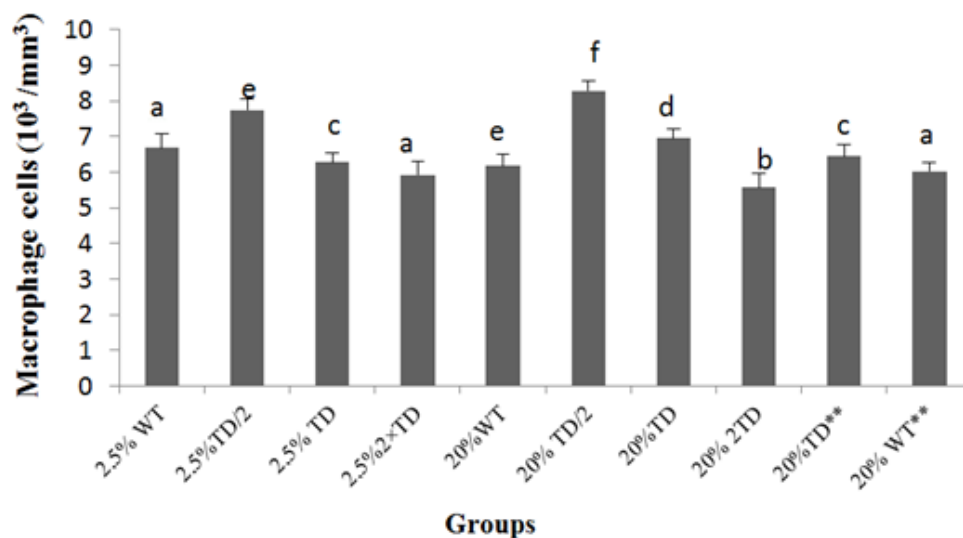
Protein energy malnutrition (PEM) is a range of pathological conditions arising from coincidental inadequate intake of in varying proportions of proteins and calories, occurring most frequently in infants and young children and commonly associated with infections (Muscaritoli *et al.*, 2009). Three types of protein-energy malnutrition (marasmus, marasmic kwashiorkor, and kwashiorkor) have been identified, described and classified (Manary *et al.*, 2009). They are characterized by low weight for age, oedema, dermatitis, hair changes, mental changes, hepatomegaly and diarrhea. The group of experimental animals that received low protein diet exhibited retarded growth, hair loss and physical inactivity thereby confirming the establishment of protein energy malnutrition (Bayoumy, 2013).

Albumin was traditionally measured to estimate the nutritional status but it has the disadvantages of having a long half-life (18-20 days) (Behar, 1981). Its major role is in the assessment of the severity of chronic malnutrition and in estimating prognosis (Veldee, 2001). In this research, the rats that were given low protein diet showed a significant decrease in serum albumin (2.00 ± 0.35) compared to the control group (3.13 ± 0.90 g/l) confirming the establishment of severe protein energy malnutrition.

Anaemia is one of the common complications of protein-energy malnutrition (Borelli *et al.*, 2007). Moreover, suppression of cell mediated immunity is something that is typically found in almost all patients with protein energy malnutrition (Nesdyaningtyas & Firani, 2012; Kew *et al.*, 1999). The results of this finding showed a significant decrease in red blood cells concentration and pack cell volume (PCV) in the protein malnourished rats, expressing the establishment of anemia in them. Furthermore,

they also highlight leucopenia and thrombocytopenia with increased neutropenia and lymphocytic infiltration leading to an increased DTH in malnourished rats. Indeed, when the amount of proteins consumed is less than the requirement it leads to a reduction in cell mediated immunity, and consequently the number of lymphocytes is also reduced. The formation of various immune systems cells in the body depends of the body's ability to synthesize proteins. Malnutrition is therefore an important risk factor for infectious diseases, because cell-mediated immunity is the key host defense against microorganisms.

volume, red blood cells and white blood cells concentration values of malnourished rats that received *S. abyssinica* leaves powder as supplement of low protein diet or normal protein diet were not affected upon 28 days of treatment. Moreover, upon rehabilitation the protein malnutrition induced group of experimental animals were characterized by a restoration to normal dermal conditions, loss of edema, hair growth, noticeable restoration to normal physical activities, motor co-ordination, and weight gain. This evidence shows the great nutritional potential of *S. abyssinica* to support growth and rehabilitation of protein energy malnutrition

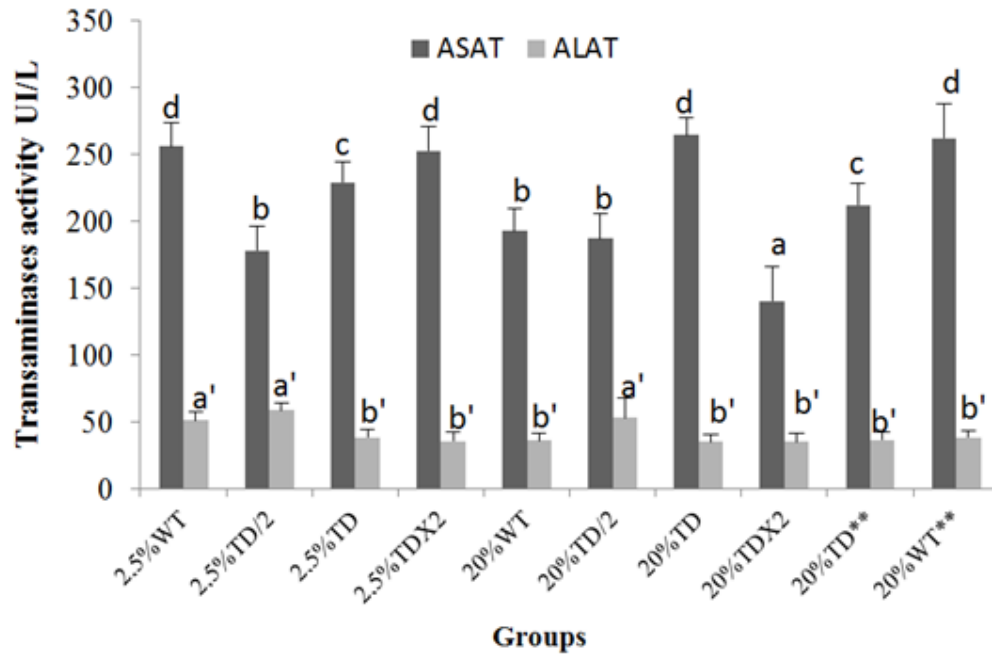


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Figure 6: Effect of *S. abyssinica* leaves powder supplement on peritoneal macrophages mobilization

The use of *S. abyssinica* powder to correct the malnourished state can thus be justified. The results showed that the serum albumin, pack cell

subjects, and corroborates the findings of [Mosha & Bennink \(2004\)](#).



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Figure 7: Effect of *S. abyssinica* dry leaves powder supplement on serum transaminases

The nutritional quality of a protein food depends on the content, rates of digestion, absorption, and utilization of amino acids. Availability of amino acids varies with protein source, food processing treatments, and interaction with other components of the diet. *S. abyssinica* dry leaves significantly improved the growth performance. These leaves may contain appreciable amount of amino acid essential for rat development.

Previous study has shown that *S. abyssinica* leaves contain various ions such as iron, zinc, vitamins and their haematinic property is well known (Osolo *et al.*, 1996), and may justify the haematinic properties observed. Result of this finding is in line with earlier reports of

haematinic potential of plants (Agbor & Odetola, 2001; Alada, 2000).

Borelli *et al.* (1998) and Mc Carter *et al.* (1998) have reported that protein deficiency tend to decrease the number of lymphocytes and functions of T- helper cells, natural killer cells, and peritoneal macrophages. Decline in the number of lymphocytes is also associated with a lower intake of the amino acid glutamine in the diet. Lymphocytes require glutamine, both in un-activated and activated conditions by mitogen Kew *et al.*, (1999). The delayed-type hypersensitivity (DTH) reaction is an indicator of T-cell mediated immunity (Kanjwani *et al.*, 2008).

The *S. abyssinica* leaves powder significantly increased the number of immune cell at the peritoneal cavity as a response of ovalbumin and reduced the DTH response. These evidences highlight the immunostimulatory property of the plant which could be of immense benefit as a dietary supplement to alleviate immune deficiency due to protein-energy malnutrition.

Protein-energy malnutrition is known to be associated with many biochemical disturbances in the body. The supplementation of the two diets with *S. abyssinica* significantly reduced the activity of transaminases, indicating that *S. abyssinica* leaves powder restored the function of liver during protein-energy malnutrition treatment. Indeed, the increase in serum alanine aminotransferase and alkaline phosphatase in animals fed with protein deficient diet suggests hepatocellular dysfunctions (Obatolu *et al.*, 2003).

The *S. abyssinica* leaves was found to overcome all the clinical characteristics induced by low protein intake independent of the diet from 110 kg b.w. indicating the importance of this plant in protein-energy malnutrition conditions either as prophylaxis or dietary therapy. This result raised up more interest, since the correction of protein-energy malnutrition could be achieved with low protein diet conditions. The result are relevant since this plant could be used in various regions of Cameroon and all over the world where many populations live in extreme poverty leading to the development of such disease in infant and young children.

4. Conclusion

The results of this finding clearly demonstrate that *Stephania abyssinica* leaves powder has a significant nutritional quality that contributes to ameliorate the growth performance and promote

good biological responses in protein manutrition induced rats. They could serve as prophylaxis and dietary therapy of protein energy malnutrition.

Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

All animal care and experimental protocols were ethically reviewed and approved by the Department of Biochemistry, Faculty of Science, University of Dschang, Cameroon. This study does not involve any human testing.

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