Research Article

INFLUENCE OF PROBIOTICATED *MORINGA OLEIFERA* LEAF EXTRACT FOR TREATMENT OF ANAEMIA USING ANIMAL MODEL

S. Anita^{1*}, M. Aravindh² and E. Ramya²

¹Department of Food Technology, K. S. Rangasamy College of Technology, Tiruchengode, Namakkal District, Tamil Nadu, India

²Department of Biotechnology, K. S. Rangasamy College of Technology, Tiruchengode, Namakkal District, Tamil Nadu, India

*Corresponding Author Email: anita.sundar@gmail.com

Article Received on: 26/04/17 Approved for publication: 20/05/17

DOI: 10.7897/2230-8407.08582

ABSTRACT

An attempt was made to evaluate anti-anaemic properties of probiotic *Moringa oleifera* leaf extract using animal model. Twenty four male rats were randomly assigned to four groups and all the animals were given Phenylhydrazine orally to induce anaemia. *Enterococcus durans* was selected as probiotic supplement. Group 1 was provided with 300mg/kg of *M.oleifera* leaf extract. Group 2 was supplemented with 0.3 ml of *E.durans* along with 300mg/kg of *M. oleifera* leaf extract. Group 3 and 4 were fed with *E.durans* and iron supplement (1ml/rat) respectively. Blood samples were collected on a weekly basis and various haematological and biochemical parameters were analysed. The haemoglobin level was found to be higher with 14.9 ± 0.25 g/dl for rats fed with probioticated *M.oleifera* leaf extract. *E.durans* has proliferated in gut which was verified through faecal and gut microbiota analysis. Hence, probiotic *M.oleifera* leaf extract was found an effectual nutraceutical supplement to treat anaemia.

Key words: Anaemia, *Moringa oleifera*, *Enterococcus durans*, phenyl hydrazine, Albino wistar rat.

INTRODUCTION

Nutrition is a nucleus of human development and major source for meeting the nutritional requirements is healthy food. However, prevalence of nutritional deficiency is increasing enormously and among them, anaemia has become life threatening disease. About 30% of the world's population is affected due to iron deficiency¹. Anaemia is characterized by drop off haemoglobin level less than 13g/dl in male and 12g/dl in female². Even though iron deficiency is supposed to be the most common cause of anaemia, other factors such as folate and vitamin B12 deficiencies, acute and chronic inflammation, parasitic infections and acquired disorders which affect haemoglobin synthesis and RBC production leads to anaemic condition³. Oral iron therapy remains challenging due to long term treatment and gastrointestinal adverse effects⁴. In the last few years, a promising development in the field of nutraceuticals and functional foods was perceived. Several herbal based nutraceutical products are available in market to prevent chronic diseases. *Moringa oleifera*, belonging to the family Moringaceae is one such plant which grow fast and found to be widely distributed in India⁵. The plant is renowned for its medical properties⁶ and it is used as antispasmodic, stimulant, expectorant, antifertility, anticancer, hepatoprotectivity, antiulcer, and diuretic agent⁷. The extracts of *M.oleifera* leaf have been proven to have potent antioxidant activity⁸. *M.oleifera* leaves apart from improving haemoglobin levels acts as excellent source of all essential amino acid, iron, calcium, potassium and vitamin c. Incorporating the *M.oleifera* leaf in regular diet could alleviate micronutrient deficiencies and many products such as *Moringa* tea, tablets, capsules are been formulated⁹.

Probiotics are live microorganisms which when ingested in certain quantity exert health benefits to the consumers beyond inherent general nutrition. Exposure to antibiotics, infection and dietary changes leads to alteration in microbiome - host symbiosis and cause impairment to health-promoting bacteria¹⁰. This can be overcome by regular intake of probiotic supplements in diet. Probiotic microbiota plays a key role in the improvement and functionality of the innate and adaptive immune responses¹¹. In addition to the health benefits credited to probiotics due to colonization, growth and activity in the gut, these species may possibly increase iron bioavailability either through fermentation of the food products or arresting the growth of pathogenic bacteria. Hence, formulation of novel nutraceutical products using *M.oleifera* leaf extracts and probiotic bacteria for treatment of anaemia could be more favourable and suitable resource for recovery of anaemia. With necessities and demands for these kind of products are being increasing in pharmaceutical industries, the present research aims to evaluate the haematological parameters, biochemical analysis and gut microbiota of Wistar rats supplemented with probioticated *M. oleifera* leaf extract for treatment of anaemia.

MATERIALS AND METHODS

Preparation of *M.oleifera* leaves extract and probiotication process

M.oleifera leaves were collected from Erode district, Tamilnadu and it was authenticated by Department of Biotechnology, K.S.Rangasamy College of Technology. Leaves were washed after soaking in 1% NaCl solution for 5 minutes and it was shade dried at room temperature. The dried leaves were milled and sieved to fine powder and stored in air tight container for further

analysis⁹. Leaf extract was prepared by macerating 200gm of dried powder in ethanol for 48 hrs. This was filtered with Whatman No. 1 filter paper and percolated extract was then dried in rotary evaporator. The powdered leaf extract were further rehydrated and diluted to necessary concentration before initiation of treatment. Probiotic bacteria were isolated from curd and it was identified as *Enterococcus durans* through molecular characterization. Further, *M.oleifera* leaf extract was probioticated through addition of 2% of *E. durans* followed by incubated at 37° C for 72 h.

Grouping of experimental rats

Ninety-days-old Wistar rats weighing 150-250 grams were obtained from the animal house of Department of Biotechnology, K.S.Rangasamy College of Technology. Experimental design involves four groups with six animals in each treatment with an average weight difference of $\leq 1.3\text{g}$. The animals were maintained in individual clean plastic cages under the temperature $20\pm 2^\circ\text{C}$ and humidity ($50\pm 5\%$) with 12 hours light/dark cycles and were allowed access to standard commercial feed pellets and clean water was provided *ad libitum* throughout the experiment periods. Rats with Hb content of above 14 g/dl were selected for the present investigation. Treatment details include, Group 1: Supplemented with *Moringa oleifera* leaf extract (300mg/kg body weight). Group 2: Supplemented with probioticated *Moringa oleifera* leaf extract ($10^7\text{CFU}/0.3\text{ ml}$ with 300 mg *Moringa oleifera* leaf extract/kg body weight). Group 3: Supplemented with Lactic acid bacteria ($10^7\text{CFU}/0.3\text{ ml}$ / kg body weight) and Group 4: Supplemented with commercially available iron supplements of 1ml/rat¹². The dose was administrated to all the rats via gavage and amount of dose given to each rats was calculated individually based on body weight. All the experiments on animals were approved by Institutional Animal Ethics Committee (IAEC), New Delhi, with ethical clearance number KSRCT/BT/IAEC/2015/01, prior to the commencement of the experiments.

Induction of anaemia

Before induction of anaemia, initial Hb concentration was determined through collecting blood from tail vein of each animal. Further, anaemia was induced to all the experimental rats by continuous oral administration of Phenylhydrazine at 10mg/kg body weight for 8-10 days¹³. Haematinic effects can be studied through induction of Phenylhydrazine in animal model¹⁴.

Body weight analysis

Body weight of individual experimental rats was measured at weekly intervals till the end of the experiment and percentage of increase was evaluated through method outlined by Nku-Ekpang et al.¹⁵.

Haematology studies

The blood samples were collected from all the experimental animals on a weekly basis by tail vein method and the collected blood was immediately stored in EDTA coated tubes. The haematological parameters such as Haemoglobin, RBC, WBC, Platelets, Neutrophils, Eosinophils, Basophils, Monocytes and Lymphocytes were analysed using Haematology analyser.

Biochemical analysis

The blood samples were collected from all the experimental rats, centrifuged for 10 minutes at 5000 rpm. The serum was separated

and used for further tests. Various biochemical analyses such as glucose, triglycerides, protein, albumin, globulin, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase was studied using automated Biochemical analyser.

Microbial analysis

The probiotic efficiency of LAB in faecal samples and various segments of GI tract such as ileum, stomach and rectum was enumerated using spread plate method¹⁶. The faecal samples were collected at weekly intervals from each rat before administration of the sample for microbial analysis. After 30 days, all the animals were euthanized with increased concentration of Phenobarbitone and their organs were subjected to analysis of microbiota. All the organs were sliced approximately to 1cm length fragment and placed into MRS broths and kept overnight at room temperature for the proliferation. Further, the broth was serial diluted and were spread plated in MRS medium and incubated for 24 to 48 hours at room temperature. Translocation assay was performed through the method illustrated by Vincent et al.¹⁷. The organs of rats (heart, kidney, liver) were obtained after scarification of the animal under sterile conditions. The organs were chopped approximately to 1cm length fragment and incubated into MRS broths and kept overnight at room temperature. The next day, the broth was serial diluted upto 10^{-8} concentrations and were spread plated in MRS medium and incubated for 24 to 48 hours at room temperature. The growth of prominent cultivable microflora was enumerated on the basis of colony-forming units (cfu).

Statistical analysis

Statistical analysis was implemented by ANOVA followed by Duncan's multiple range test (DMRT) using Statistical Package for Social Science (SPSS) version 17.0. Results were expressed as mean $\pm$ SD for six rats in each group.

RESULTS AND DISCUSSION

Body weight analysis

Anaemia is considered to be one of the most predominant nutritional problems in recent years¹⁸. The present study aims in developing probiotic based *M.oleifera* herbal extract against Phenyl hydrazine induced anaemia in Wistar rats. Figure. 1 illustrates the effect of different treatments on increase in percentage of body weight of rats. Comparing to first week trial, all the rats invariably showed steady increase in body weight with significant difference ($p<0.05$). At end of the fourth week, rats fed with probiotic *M.oleifera* leaf extract gave 26.4% increase in body weight and it was on par with group 3 rats fed with probiotic bacteria alone. On the other hand, group 1 and 4 gave almost similar increase in percentage of body weight with no significant difference. *M.oleifera* leaf extract combined with probiotic bacteria have additional benefits in suppressing the growth of pathogen which concurrently enhancing the absorption of nutrient and could act as vital growth promoters. This study confirm the earlier feeding trails carried out for albino rats fed with encapsulated *Lactobacillus* spp. along with *M.oleifera* leaf powder has increased the weight from 150gm to 230gm with approximate 30% weight gain¹⁹. A significant weight gain was observed in rats fed with *L.plantarum*²⁰. Nku-Ekpang et al.,¹⁵ observed mean body weight increases with increased dose of ethanolic extract of *M.oleifera* for Phenylhydrazine challenged anaemic wistar rats.

Table 1: Effect of different treatments on haemoglobin and RBC levels in the blood of experimental rats

Duration	Treatments							
	Group 1		Group 2		Group 3		Group 4	
	Hb (g/dl)	RBC 10 ⁶ /mm ³	Hb (g/dl)	RBC 10 ⁶ /mm ³	Hb (g/dl)	RBC 10 ⁶ /mm ³	Hb (g/dl)	RBC 10 ⁶ /mm ³
Initial	14.5 ^a ±0.24	8.51 ^a ±0.24	14.2 ^a ±0.64	8.45 ^a ±0.34	14.5 ^a ±0.23	8.42 ^a ±0.38	14.2 ^a ±0.15	8.56 ^a ±0.14
Week 1	10.7 ^a ±0.45	6.35 ^a ±0.34	10.6 ^a ±0.34	6.17 ^a ±0.13	10.8 ^a ±0.62	6.26 ^a ±0.43	10.7 ^a ±0.22	6.32 ^a ±0.17
Week 2	11.3 ^a ±0.61	6.6 ^a ±0.28	11.5 ^a ±0.26	6.63 ^a ±0.15	11.3 ^a ±0.72	6.5 ^a ±0.38	11.7 ^a ±0.17	6.35 ^a ±0.13
Week 3	12.6 ^a ±0.63	6.92 ^a ±0.15	12.8 ^a ±0.15	6.91 ^a ±0.22	12.3 ^a ±0.38	6.92 ^a ±0.28	12.8 ^a ±0.11	6.77 ^a ±0.26
Week 4	14.0 ^{bc} ±0.08	8.15 ^a ±0.06	14.9 ^a ±0.25	8.15 ^a ±0.1	14.2 ^{bc} ±0.18	8.00 ^a ±0.12	14.3 ^b ±0.21	8.12 ^a ±0.17
% of improvement	30.8	22.08	40.5	24.29	31.8	27.75	33.6	22.16

Values represent average ± SD; similar alphabets followed in a row denote they are statistically insignificant and vice versa at $P < 0.05$.

Table 2: Effect of different treatments in the haematological parameters of experimental rats

Haematological parameters	Group 1	Group 2	Group 3	Group 4	Reference Value
WBC (10 ³ /mm ³)	8.90 ^a ±0.14	9.02 ^a ±0.09	8.92 ^a ±0.32	8.73 ^a ±0.09	4.5 to 11
Platelets (10 ³ /mm ³)	423.33 ^a ±59	434.23 ^a ±24	400.33 ^a ±66	410.75 ^a ±21	150 - 450
Neutrophils (%)	16.55 ^a ±0.66	16.55 ^a ±1.00	16.57 ^a ±0.74	16.97 ^a ±0.91	8-24
Eosinophil (%)	0.12 ^a ±0.05	0.22 ^a ±0.08	0.22 ^a ±0.06	0.11 ^a ±0.04	0-4
Basophils (%)	0.05 ^a ±0.01	0.0 ^a ±0.0	0.01 ^a ±0.08	0.04 ^a ±0.06	< 0.1
Monocytes (%)	0.77 ^a ±0.56	0.89 ^a ±0.21	0.97 ^a ±0.62	0.60 ^a ±0.41	1-6
Lymphocytes (%)	75.77 ^a ±1.20	77.9 ^a ±1.89	79.07 ^a ±1.96	76.65 ^a ±1.71	70-89

Values represent average ± SD; similar alphabets followed in a row denote they are statistically insignificant and vice versa at $P < 0.05$.

Table 3: Effect of different treatments on biochemical parameters of phenyl hydrazine induced anemic rats

Parameters	Treatments				Reference value
	Group 1	Group 2	Group 3	Group 4	
Glucose (mg/dl)	87.30 ^{bc} ±3.27	109.94 ^a ±5.4	92.21 ^{bc} ±4.55	86.73 ^{bc} ±3.73	80-120
Triglycerides (mg/dl)	67.65 ^a ±0.46	65.23 ^b ±1.55	67.15 ^a ±0.55	68.38 ^a ±3.13	65-170
Protein (g/dl)	7.38 ^a ±0.29	7.71 ^a ±0.31	7.57 ^a ±0.1	7.34 ^a ±0.73	6.0 – 8.0
Albumin (g/dl)	3.72 ^a ±0.01	3.93 ^a ±0.06	3.82 ^a ±0.08	3.57 ^a ±0.25	3.7 – 5.3
Globulin (g/dl)	3.60 ^a ±0.42	3.62 ^a ±0.08	3.70 ^a ±0.13	3.19 ^a ±0.37	2.3-3.8
Urea (mg/dl)	15.89 ^c ±0.77	24.66 ^a ±2.73	20.50 ^b ±1.6	18.93 ^{bc} ±1.44	13-43
Creatinine (mg/dl)	0.48 ^c ±0.04	0.45 ^c ±0.02	0.89 ^{ab} ±0.01	0.95 ^a ±0.05	0.6-1.1
ALT (U/L)	46.46 ^a ±1.29	49.62 ^a ±1.00	47.51 ^a ±1.76	44.69 ^a ±2.54	13- 66
AST (U/L)	31.38 ^{bc} ±0.77	38.82 ^a ±2.01	34.11 ^{ab} ±1.31	29.08 ^c ±3.48	≤ 40
ALP (U/L)	245.65 ^a ±3.49	263.45 ^a ±14.79	258 ^a ±4.33	256.04 ^a ±9.64	≤ 280

Values represent average ± SD; similar alphabets followed in a row denote they are statistically insignificant and vice versa at $P < 0.05$.

Figure 1: Effect of different treatments on percentage increase in body weights of experimental rats

Wistar rats fed with *M.oleifera* leaf extract (I), probiotic *M.oleifera* leaf extract (II), probiotic (III) and commercial iron supplementation (iv) during 30 days trial. Values represent average $\pm$ SD. Bars represent standard error values.

Figure 2: Enumeration of Lactic acid bacteria in faecal sample of experimental rats

Wistar rats fed with *M.oleifera* leaf extract (I), probiotic *M.oleifera* leaf extract (II), probiotic (III) and commercial iron supplementation (iv) during 30 days trial. Bars represent standard error values.

Figure 3: Enumeration of Lactic acid bacteria counts of stomach (A), ileum (B) and rectum (C) of experimental rats

Wistar rats fed with *M.oleifera* leaf extract (I), probiotic *M.oleifera* leaf extract (II), probiotic (III) and commercial iron supplementation (iv) during 30 days trial. Bars represent standard error values.

Haematology studies

The effects of supplementation of probiotic *M. oleifera* leaf extract on Hb and RBC content of anaemic rats were presented in Table 1. According to Unami²¹, Hb content below 14g/dl was considered to be anaemic for rat models. Oral induction of Phenyl Hydrazine has reduced Hb content severely and occurrence of haemolytic anaemia was confirmed with rapid decrease of Hb to an average of 10.7 g/dl (74.56%). Phenyl hydrazine is a strong oxidizing agent and it oxidise RBC and causes severe haemolytic anaemia²². Hb content restored to normal level after 30 days with drastic improvement of 40.5% for group 2 rats which was fed with probioticated *M. oleifera* leaf extract with vast significant difference ($p < 0.05$) when compared to other groups.

On the other hand, no significant difference was observed between group 1, 3 and 4 which showed 30.8, 31.8 and 33.6% of increased in Hb content respectively. Similarly, impact of administration of Phenyl Hydrazine has reduced the RBC counts to an average of 26% for all the groups. However, after initiation of treatment gradual increase in RBC counts was noticed without any significant difference within the groups. At end of the treatment, group 1 and 2 showed similar RBC count of $8.15 \times 10^6/\text{mm}^3$ followed by $8.12 \times 10^6/\text{mm}^3$ RBC count for rats supplemented with iron tonic. Surprisingly, Group 3 rats which received only probiotic dose also gave almost similar value of $8.00 \times 10^6/\text{mm}^3$ of RBC count without any significant difference between other treatments. *M.oleifera* upsurges the blood cell production and could treat haemolytic and haemorrhagic anaemia¹⁵.

Table 2 illustrates the various haematological parameters for the different groups of treatments. All the parameters were found to be within the normal range²³. An increase in WBC counts was observed for experimental rats fed with probiotic *M.oleifera* leaf extract with $9.02 \times 10^3/\text{mm}^3$ with no significant difference when compared to other treatments. The value of platelets showed a significant increase with probiotic *M.oleifera* extract and *M.oleifera* extract alone at $434.23 \times 10^3/\text{mm}^3$ and $423.33.75 \times 10^3/\text{mm}^3$ respectively. No significant difference was observed for other haematological indices such as Neutrophils, Eosinophils Basophils and Monocytes. Probiotics retain immune modulatory properties and hence Lymphocytes count was slightly higher in group 2 and group 3 rats as they were fed with *E.durans*. Pathogenicity which leads to infection is the most notable criteria which have to be assessed in probiotic studies to ensure safety²⁴. Occurrence of bacterial infection can be detected through increase of Neutrophils count in blood samples²⁵.

The present study confirms the absence of inflammation in the all experimental rats through normal level of Neutrophils, Eosinophil and Basophils counts. The present study highlights the work done by de Azerêdo et al.²⁴ that, mice receiving *Z. mobilis* as probiotic supplements did not detected higher levels of neutrophils counts and experienced no infection from the treatment. Oral ingestion of Lactic acid bacteria in Wistar rats increases lymphocyte proliferation and interferon production which relates to immuno - stimulatory effect²⁶. This highly correlates the present research that level of lymphocyte was found to be increased in group 2 and 3 rats which received probiotic supplements.

Biochemical analysis

The toxicological results of probiotic *M.oleifera* leaf extract and other treatments on biochemical parameters were illustrated in the Table 3. It was interesting to note that, all parameters were found to be normal for all the groups. Fasting glucose level of 109.94

mg/dl was reported for group 2 rats and it was within the reference range for all the treatments. Triglycerides were found to be lower with 65.23 mg/dl whereas serum urea level was found to be slighted higher with 24.66 mg/dl for group 2 rats with significant difference. *M.oleifera* leaf extracts exhibit hypocholesterolemic effect²⁷. Previous studies conducted by Zavisic et al.,²⁸ reported that supplementation of probiotic bacteria *L.plantarum* and *L.casei* to Wistar rats has reduced serum cholesterol and triglycerides than compared to control rats. This highly correlates with the present work while triglycerides values were found to lower for rats fed with probiotic *M.oleifera* leaf extracts than compared to other treatments. It might be due to the combined activity of probiotic bacteria and *M.oleifera* leaf extract. Rabbits fed with *Moringa oleifera* leaf meal did not show any have adverse effect on the biochemical response²⁷. Other parameters such as total protein, albumin and globulin content were found to be almost similar with no significant variation. *M. oleifera* leaf extract can substantially decrease high creatinine concentration in serum.

However, serum creatinine level showed negligible variation with insignificant results. The enzyme pattern of ALT, AST and ALP were found to be within the normal range for all the treatment. ALT and ALP gave no significant variations between the groups whereas, group 2 rats showed substantial increase in AST followed by group 3 rats. Normal level of Serum urea, creatinine and enzymes such as ALT, AST and ALP levels reveal proper function of kidney and liver in rats. Hence, probiotic bacteria and *M.oleifera* leaf extract supplements were safety to use and had no side effects. These results were in agreement with work of Abd El-Moneim et al.,²⁹ were the safety of probiotic biscuits was confirmed through liver and kidney function were significant change was not observed in serum ALT, AST, ALP, creatinine and urea. Thomas and Lee³⁰ recorded insignificant difference in ALT between the rats fed with LAB capsule and control.

Microbial analysis

Adhesion of supplemented probiotic bacteria to the host's GI tract is crucial importance due to its ability to trigger the host's immune response³¹. Hence, ability of *E.durans* to adhere and colonise the GI tract mucosa of rats has been investigated through faecal and gut microbial analysis (Figure 2 and 3). A significant growth of LAB was observed in samples of Group 2 and 3 on 7th and 14th day treatment. Group 1 rats fed with *M.oleifera* leaf extract alone showed average increase in growth of probiotic bacteria.

However, rats fed with commercial iron tonic supplements showed poor growth of probiotic bacteria than compared to other treatments. The present study support the work of Kumalaningsih et al.¹⁹ whereas, the addition of *M.oleifera* leaf extract increases in the growth of Lactic acid bacteria.

No abnormal clinical signs were observed for the groups supplemented with *E.durans* during the experimental period and proliferation of LAB in the different segments of GI Tract such as stomach, ileum and rectum were illustrated in Figure 3. The results were highly correlated with enumeration of LAB from faecal samples. Growth of LAB was found to be predominant in ileum of group 2 rats and stomach region of group 3 rats and no significant difference was observed. Surprisingly, remarkable LAB growth was noticed in ileum region of group 1 rats. Translocation assay for all the groups gave positive results and no microbial growth was observed in organs like Heart, Kidney and Liver. The results of translocation assay validate that safety usage of *E.durans* as an effective probiotic strain.

In summary, development of new probiotic based functional product is essential to assured for safety and to be proved for absence of pathogenicity³². Hence, present research emphasis was on safety and its combined effect in improving the nutritional status and particularly haematological functions of experimental rats. Several studies have highlighted the usage of *M.oleifera* leaf extract and probiotic bacteria as a separate product for health benefits.

The present research discloses that, haematinic activity of probiotic *M.oleifera* leaf extract was found to be superior when compared to *M.oleifera* leaf extract, probiotic bacteria and commercial iron supplements. Henceforth, *M.oleifera* leaf extract along probiotic bacteria could act as iron supplements to cure anaemia.

ACKNOWLEDGEMENT

We acknowledge DST-FIST for providing the necessary laboratory facility and our special thanks to Lion Dr.K.S. Rangasamy, Chairman, Dr. K. Thyagarajah, Principal, and Dr.P. Ponmurugan, Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode, Tamil nadu, India, for providing facilities and encouragement.

REFERENCES

1. Aattouri N, Bouras M, Tome D, Marcos A, Lemonier D.: Oral ingestion of lactic acid bacteria by rats increases lymphocyte proliferation and interferon gamma production. Br Journal of Nutrition 2001; 87:367-73.
2. Abd El-Moneim MRA, Ramy MR, Shaimaa IMS, Mona MH: Antioxidant activity and biological evaluations of probiotic bacteria strains. International Journal of Academic Research 2012;4:131-139
3. Aboderin FI, Oyetayo VO.: Haematological studies of rats fed different doses of probiotics, *Lactobacillus plantarum*, isolated from fermenting corn slurry. Pakistan Journal of Nutrition 2006;5 : 102-105.
4. Agbor GA, Oben JE, Ngogan JY.: Haematinic activity of *Hibiscus cannabinus*. African Journal of Biotechnology 2005;15:833-837.
5. Ai Zhao, Yumei Zhang, Ying Peng, Jiayin Li, Titi Yang, Zhaoyan Liu, Yanli LV, Peiyu Wang.: Prevalence of Anemia and Its Risk Factors Among Children 6-36 Months Old in Burma. American Journal of Tropical Medicine and Hygiene 2012; 87:306-31.
6. Ashish T, Sachin M, Rizwan SJ, Gadhpayle SL, Bhongade: Inhibitory potential of *Picrorhiza kurroa royle* ex. Benth extracts on phenylhydrazine induced reticulocytosis in rats. Asian Journal of Pharmaceutical and Clinical Research 2013; 6:215-16.
7. Ayaz S, Usmanghani K, Nazar H.: Alternative treatment for iron deficiency anemia: Irobin versus sherbat Faulad. Pak Journal of Pharmacology Science 2016; 29:157-64.
8. Berger J.:Phenylhydrazine haematotoxicity. Journal of Applied Biomed 2007; 5:125-30.
9. Berger J.: Screening of toxic-haemolytic anaemia in laboratory rats: a model of Phenylhydrazine induced haemolysis. Haematologia 1985; 18:193-200.
10. Cameron DG, Watson GM.: The blood counts of adult albino rat. Blood Journal 1949; 4: 816-818.
11. Cryan JF, Dinan TG.: Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nature Reviews Neuroscience 2012; 13:701-12.
12. De Azerêdo GA, Stamford TLM, de Souza EL, Veras FF, de Almeida ER, de Araújo JM.: *In vivo* assessment of possible probiotic properties of *Zymomonas mobilis* in a Wistar rat model. Electronic Journal of Biotechnology 2010; 13: 3-4.
13. Ewuola EO, Jimoh OA, Atuma OV, Soipe OD.: Haematological and serum biochemical response of growing rabbits fed graded levels of *Moringa oleifera* leaf meal. In: Abstracts: Proceedings 10th World Rabbit Congress, Sharm El- Sheikh, Egypt 2012; 679-683.
14. Garima M, Pradeep S, Ramesh V, Sunil K, Saurabh S, Jha KK, Khosa RL.: Traditional uses, phytochemical and pharmacological properties of *Moringa oleifera* plant; An overview. Der. Pharmacia. Letter 2011; 3:141-64.
15. Iskandar I, Hadju V, As'ad S, Natsir R.: Effect of *Moringa oleifera* Leaf Extracts Supplementation in Preventing Maternal Anemia and Low-Birth-Weight. International Journal of Scientific and Research Publications 2015;5(2):1-3
16. Kumalaningsih S, Padaga M, Suprayogi, Vitta Rizky P.: Encapsulation of *Lactobacillus* sp. with *Moringa Oleifera* leaves extract for food supplementation. International Research Journal of Agriculture Science and Soil Science 2011; 1: 273-77.
17. McLean E, Cogswell M, Wojdyla D, de Benoist B.: Worldwide prevalence of anaemia. WHO Vitamin and Mineral Nutrition Information System (VMNIS). Public Health Nutrition 2009;12(4):444-54.
18. Mishra SP, Singh P, Singh S.: Processing of *Moringa oleifera* leaves for Human consumption. Bulletin on Environmental Pharmacology Life Science 2012; 2: 28-31.
19. Nku-Epkang Okot-Asi T, Nwaehujor Chinaka O, Ofem Ofem E, Ezekiel Josiah I.: Effect of *Moringa oleifera* Lam. Ethanol leaf extract on Hematology in Phenylhydrazine-induced Anaemic Albino Wistar rats. American Journal of Pharmacological Sciences 2015; 3:67-73.
20. Ouwehand AC, Salminen S, Isolauri E.: Probiotics: an overview of beneficial effects. Antonie Van Leeuwenhoek 2013; 82:279-89.
21. Panda DS, Choudhury NSK, Yedukondalu M, Si S, Gupta R.: Evaluation of Gum of *Moringa oleifera* as a Binder and Release Retardant in Tablet Formulation. Indian Journal of Pharmacology Science 2008; 70(5):614-18.
22. Perhar R, Shukla R, Mishra N.: Management of Iron Deficiency Anaemia-Recent Challenges. Paripex - Indian Journal of Research 2015; 4(2):1-3.
23. Rescigno M.: The intestinal epithelial barrier in the control of homeostasis and immunity. Trends in Immunology 2011; 32: 256-264.
24. Ruckmani K, Kanimani S, Anandan R, Jaykar D.: Effect of *Moringa oleifera* Lam on paracetamol – induced hepatotoxicity. Indian Journal of Pharmacology Science 1998;60(1):33-35.
25. Saarela M, Mogensen G, Fonden R, Matto J, Matilla ST.: Probiotic bacteria: Safety, functional and technological properties. Journal of Biotechnology 2000;84:197
26. Thomas P, Lee SH. The safety of capsules containing *Lactic acid bacilli*. Annals of Dent. University 1999; 6: 56-57.
27. Tihihonen K, Suomalainen T, Tynkkynen S, Rautonen N.: Effect of prebiotic supplementation on a probiotic bacteria mixture: comparison between a rat model and clinical trials. Brazilian Journal of Nutrition 2008; 99: 826-31.
28. Turaskar A, More S, Sheikh R, Gadhpayle J, Bhongade SL.: Inhibitory potential of *picrorhiza kurroa royle* ex. Benth extracts on phenylhydrazine induced reticulocytosis in rat. Asian Journal of Pharmacy and Clinical Research 2013; 6: 215-16.
29. Unami A, Nishina N, Terai T, Sato S, Tamura T, Noda K, Mine Y. Effect of cisplatin on erythropoietin production in rats. Journal of Toxicology Science 1996; 2: 157-165.

30. Vincent D, Roy D, Mondou F, Dery C.: Characterization of *Bifidobacterium* by random DNA amplification. International Journal of Food Microbiology 1998; 43:185-193.
  31. Zavisic G, Petricevic S, Radulovic Z, Begovic J, Natasa G, Topisirovic L, Strahinic I.: Probiotic features of two oral *Lactobacillus* isolates. Brazilian Journal of Microbiology 2012; 43:418-428.
  32. Zhou JS, Pillidge CJ, Gopal PK, Gill HS.: Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. International Journal of Food Microbiology 2005; 98:211-17.
- Cite this article as:**
- S. Anita et al. Influence of probioticated *Moringa oleifera* leaf extract for treatment of anaemia using animal model. Int. Res. J. Pharm. 2017;8(5):101-107 <http://dx.doi.org/10.7897/2230-8407.08582>

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: IRJP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.