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Comprehensive Evaluation of Malnutrition Effect on Pain Threshold in Albino Rats

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Abstract: This present study was aimed at exploring the effect of protein malnutrition and micronutrient deficiencies on pain threshold in the rat. Thirty adult male rats used were divided into five groups of six rats each, (1) normal diet ND, (2) low-protein diet LP, (3) low protein minerals and vitamin diet LPMV, (4) low protein and vitamin diet LPV and (5) low protein and mineral diet LPM. The normal diet fed rats served as the control group. Both the control and the experimental groups were fed accordingly for a total period of six weeks. Hot plate and tail-flick (thermal) and formalin (chemical) tests were applied to assess the possible effect of protein and micronutrient deficiencies on the sensitivity of the rats to the stimuli at the end of the second, fourth and sixth week. Pain threshold was markedly reduced in the malnourished rats when compared to the control group fed with the standard diet. Latencies significantly decreased in the groups LP, LPMV, LPV and LPM over the 0-90 min observation period in hot plate test and 48-55°C for tail immersion test; $p<0.001$. Also, significant decrease in latencies was observed in LPMV, LPV and LPM when compared with the LP group; $p<0.05$. In Formalin test, LP, LPMV, LPV and LPM groups in both early and late phases showed a more prolonged time with paw licking when compared to the control group; $p<0.001$. Similarly, when LPV and LPM were compared with the LP group, a significant increase in the licking time was observed; $p<0.05$, with significant level of LPMV being the highest; $p<0.001$. These results demonstrate that both protein malnutrition and micronutrients (vitamins and minerals) deficiencies are capable of overt hypersensitivity to acute and chronic pain.

Key words: Pain threshold, malnutrition, hot plate, tail-flick, formalin

INTRODUCTION

Clinical malnutrition is a heterogenous group of disorders including macronutrient deficiencies leading to body cell mass depletion and micronutrient deficiencies (Hughes and Kelly, 2006).

Malnutrition affects all age groups, but it is especially common among the poor and those with inadequate access to health education, clean water and good sanitation (Young *et al.*, 2004). It is the direct cause of about 300 000 deaths per year and is responsible for about half of all deaths in young children (Millward and Jackson, 2004), the risk of death directly correlated with the degree of malnutrition (De-Waal and Whiteside, 2003;

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Sachs and McArthur, 2005). More than 70% of children with protein-energy malnutrition live in Asia, 26% live in Africa and 4% in Latin America and the Caribbean (World Health Orgnization, 2000). Similarly, micronutrient deficiencies affect at least 2 billion people worldwide (Food and Agriculture Orgnization, 2004). Aside kwashiorkor and marasmus, various disorders associated with severe malnutrition have over the years been recognized.

Malnutrition impairs elements of adaptive and innate immunity which is important for defence against parasitic infections (Hughes and Kelly, 2006). It has also been reported to be associated with anaemia (Borelli *et al.*, 2007) and decreased osmotic fragility (Kaplay, 1984; Ramanadhan and Kaplay, 1982). Olowookere *et al.* (1990) explained the biochemical and bioenergetic implications of a defective in-vivo synthesis of mitochondrial proteins during dietary-protein depletion. Protein-energy malnutrition produces major structural and functional changes in the gastrointestinal tract and pancreas, which, in turn, may aggravate the underlying poor nutritional condition. It is associated with atrophy of the mucosa of the small bowel, leading to a loss of absorption as well as of digestion capacity (Alam *et al.*, 2003). According to Smith *et al.* (1975), malnourished animals are insulin resistant, with a diminished glucose tolerance and a lowered insulin to glucose ratio. Abnormal renal handling of body salts and water have also been reported in severe protein calorie malnutrition (Fiorotto and Coward, 1979). Lung and *mitochrondria* studies have also revealed that malnutrition gives rise to respiratory impairments (Olowookere *et al.*, 1991).

Evaluation of effects of malnutrition on Central Nervous System development in experimental animals and humans have long been addressed. It impairs brain development and has the potential for permanent adverse effect on learning and behaviour (Scrimshaw, 1998). On investigating its effect on the oxidative status of CNS structures, it was found, also, to be deleterious (Tatli *et al.*, 2007). Besides, alterations in behavioral parameters has consistently been demonstrated in early malnourished animals compared to the well fed (Almeida *et al.*, 1993; Rocinholi *et al.*, 1997). Most of the studies in this area, however, were carried out on lactating animals only, with electric shocks as the source of pain (Rocinholi *et al.*, 1997). This study utilized non-lactating rats and thermal tests as a source of pain, in view of the paucity of literature on relationship between nutritionally deficient diet consumption and pain sensitivity in adult rats.

Hence, the objective of this study is to investigate the effect of protein malnutrition and micronutrient deficiencies on pain threshold in adult rats of Wister strain.

MATERIALS AND METHODS

This study was carried out between June and July 2006 in the Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Animals

Thirty Males albino rats of Wister strain weighing between 180 and 200 g were used for the work. They were procured and kept for acclimatization before project commencement. They were fed for 4 weeks on normal rats diet (Ladokun feeds, Ibadan) prior to the start of the experiment and water *ad libitum*. The cages were kept constantly clean in order to prevent the rats from any disease; at 22±2°C and on a 12 h light/dark cycle. The rats were then divided into five groups of 6 rats per group. Group 1 consists of control rats which were fed with normal or standard diet (ND). Group 2 has rats that fed on a low protein diet (garri) supplemented with adequate minerals and vitamins (LP). Groups 3 rats fed on a low protein

diet (garri) without minerals and vitamins supplements (LPMV). Group 4 rats were fed on the low protein diet supplemented with minerals only (LPV). Group 5 has rats which were fed with the low protein diet supplemented with vitamins only (LPM). All the groups were fed accordingly for six weeks in the experiment.

Preparation of the Experimental Diets

The composition of the diets is as indicated in Table 1. The five groups were designated Group ND (21% protein with adequate minerals and vitamin mixture), Group LP (3.0% protein with adequate mineral and vitamin supplements), Group LPMV (3.0% protein without minerals and vitamin supplements), Group LPV (3.0% protein with mineral supplements only), Group LPM (3.0% protein vitamin supplements only).

The garri and groundnut used were purchased from a local market. The groundnut was roasted and the husk was removed and later milled. The garri was mixed with the groundnut, with vitamin and mineral mixture added to it in the proportionate amount. Lastly, palm oil was added to the mixture which was thoroughly mixed. The experimental diets were sprinkled with water and prepared into pellets.

Nociceptive Evaluation Tests

Hot Plate Test: The hot plate latency was measured using a modification of the original method of Eddy and Leimback (1953). The modified apparatus consists of an electric cooking plate (Saiso, Japan) with a 1500 Watts stainless steel heating element connected to a thermostat (0-400°C), a thermocouple connects the thermostat to a chrome plated drip pan. The thermocouple and the thermostat control the temperature of the hot plate within the (50-55°C). Pain sensitivity was evaluated by the response latency for paw licking on the hot plate. In order to avoid tissue damage, the maximum time the animal could spend on the hot plate was pegged at 50 sec. Response latencies were measured at 15 min intervals and the average of the results were taken.

Tail Flick Test

The tail flick latency was measured using the modification of D'Amour and Smiths (1941) tail immersion method. Each animal was gently hand-held while its tail was wholly immersed in water at 48, 49, 50, 51, 52, 53, 54 and 55°C. The latency for flicking the tail out the water was recorded with a stopwatch.

Formalin Test

The rats were acclimatized to the experimental arena for 15 min and anaesthetized with 5% halothane (Yamamoto and Yaksh, 1991). Formalin (50 μL, 0.25-5%) was injected subcutaneously into the large lateral foot pad on the plantar surface of the left hind paw. The rats were placed in a transparent rectangular plastic box with the top opened for an

Table 1: Composition of the experimental diets

	Stock diet (%)					
Treatments group	Carbohydrate	Palm oil	Vitamin mixture	Mineral mixture	Protein	Total
ND				21	100	
LP	87.9	4.0	1.1	4.0	3.0	100
LPMV	93.0	4.0	-	-	3.0	100
LPV	89.0	4.0	-	4.0	3.0	100
LPM	91.9	4.0	1.1	-	3.0	100

unobstructed view of the response to formalin injection which was measured using the weighted scores method (Coderre *et al.*, 1993). Time spent biting or licking the injected paw was recorded between 0-5 min (Early Phase) and 20-40 min (Late Phase).

Statistical Analysis

All data are presented as Means±SEM (Standard Error of Means). Significant differences in the hot plate and tail flick latencies and formalin scores were determined by students t test. A value of $p<0.05$ was regarded as significant and $p<0.001$ highly significant.

RESULTS

Hot Plate and Tail Flick Tests

In the malnourished group of animals, both the hot plate and tail flick latencies are significantly decreased at $p<0.05$ and $p<0.001$ over the observation period (0-90 min) and water temperatures (48-55°C), respectively.

Table 2 shows the effect of the different diets on the hot plate latency at the second week of feeding. LP, LPMV, LPV and LPM fed rats exhibited a significant reduction in hot plate latencies when compared with the normal (ND) fed rats. Out of the malnourished groups LPMV appeared to be most sensitive group as they had the lowest latencies.

Table 3 shows the effect of the different diets on the hotplate latency at the fourth week of feeding. All the malnourished groups exhibited a significant reduction of hot plate latency when compared with the normal (ND) fed rats. But the LPMV group demonstrated exceeding hypersensitivity among all the malnourished even up to the 90 min of the observation period.

Table 4 shows the effect of the different diets on the hotplate latency at the sixth week of feeding. The LPMV group has the lowest hot plate latency out of all the malnourished rats across the 0-90 min observation period when compared with the Normal Fed (ND) rats.

Table 2: Effect of different diets on hot plate latency in rats after second week of feeding

	Latencies						
Treatment groups	0 min	15 min	30 min	45 min	60 min	75 min	90 min
ND	11.3±1.0	11.4±1.0	10.2±0.8	11.6±1.1	8.3±1.4	6.5±1.0	5.1±0.7
LP	9.0±1.0[a]	8.6±0.4[a]	8.8±0.3[a]	8.0±0.4[a]	7.4±0.3	6.0±0.3	4.6±0.1
LPMV	6.8±0.2[b]	6.4±0.5[b]	5.7±0.4[b]	6.1±0.5[b]	5.5±0.3[a]	5.4±0.2	4.0±0.3
LPV	7.7±0.3[a]	8.0±0.4[a]	7.1±0.2[a]	6.7±0.4[b]	6.4±0.5[a]	6.8±0.3	4.5±0.2
LPM	7.1±0.3[a]	6.4±0.5[b]	6.1±0.5[b]	6.8±0.2[b]	6.2±0.4[a]	5.6±0.2	4.4±0.5

ND: Normal diet, LP: Low protein, LPMV: Low protein, minerals and vitamins, LPV: Low protein and vitamins, LPM: Low protein and minerals. Each value is Mean±SEM of six rats. Significant from the control group (ND) [a]$p<0.05$, highly significant [b]$p<0.001$

Table 3: Effect of different diets on hot plate latency in rats after fourth week of feeding

	Latencies						
Treatment groups	0 min	15 min	30 min	45 min	60 min	75 min	90 min
ND	11.3±1.0	11.4±1.0	10.2±0.8	11.6±1.1	8.3±1.4	6.5±1.0	5.1±0.7
LP	7.9±0.7[a]	6.9±0.5[b]	7.2±0.4[a]	6.7±0.3[b]	6.4±0.3[a]	6.8±0.2	3.7±0.2[a]
LPMV	4.7±1.0[b]	5.1±0.3[b]	4.4±0.3[b]	3.7±0.2[b]	3.3±0.2[b]	3.0±0.1[a]	2.4±0.2[a]
LPV	6.7±0.3[b]	7.1±0.2[a]	6.6±0.2[a]	5.8±0.3[b]	5.1±0.3[a]	4.2±0.2[a]	4.0±0.1
LPM	5.6±0.2[b]	5.8±0.5[b]	6.2±0.4[a]	5.5±0.3[b]	4.7±0.2[a]	3.7±0.2[a]	3.4±0.2[a]

ND: Normal diet, LP: Low protein, LPMV: Low protein, minerals and vitamins; LPV: Low protein and vitamins, LPM: Low protein and minerals. Each value is Mean±SEM of six rats. Significant from the control group (ND) [a]$p<0.05$, highly significant [b]$p<0.001$

Table 5 shows the effect of the different diets on the tail flick latency at the second week of feeding. LP, LPMV, LPV and LPM fed rats exhibited a significant reduction in tail flick latencies when compared with the normal (ND) fed rats. LPMV appeared to be most sensitive group as they had the lowest latencies, as observed in the hot plate latency.

Table 6 shows the effect of the different diets on the tail flick latency at the fourth week of feeding. Like Table 4, there is a significant reduction in the tail flick latency in the malnourished groups when compared with the normal (ND) fed rats with no significance among the LPMV, LPV and LPM group.

Table 7 shows the effect of the different diets on the tail flick latency at the sixth week of feeding. There is a significant reduction in the tail flick latency in the malnourished groups

Table 4: Effect of different diets on hot plate latency in rats after sixth week of feeding

	Latencies						
Treatment groups	0 min	15 min	30 min	45 min	60 min	75 min	90 min
ND	11.3±1.0	11.4±1.0	10.2±0.8	11.6±1.1	8.3±1.4	6.5±1.0	5.1±0.7
LP	5.3±0.4^{b}	5.3±0.2^{b}	5.5±0.6^{b}	5.8±0.4^{b}	5.4±0.4^{a}	5.6±0.4	3.3±0.2^{a}
LPMV	2.0±0.1^{b}	2.1±0.1^{b}	1.5±0.1^{b}	1.6±0.1^{b}	1.7±0.1^{b}	1.3±0.6^{b}	1.1±0.1^{a}
LPV	4.8±0.1^{b}	5.0±0.2^{b}	5.4±0.2^{b}	5.5±0.3^{b}	5.0±0.2^{a}	3.5±0.3^{a}	3.0±0.3^{a}
LPM	4.1±0.2^{b}	4.2±0.1^{b}	4.8±0.2^{b}	5.2±0.2^{b}	4.3±0.2^{a}	2.5±0.1^{a}	2.0±0.8

ND: Normal diet, LP: Low protein, LPMV: Low protein, minerals and vitamins, LPV: Low protein and vitamins; LPM: Low protein and minerals. Each value is Mean±SEM of six rats. Significant from the control group (ND) $^{a}p<0.05$, highly significant $^{b}p<0.001$

Table 5: Effects of different diets on tail-flick latency in rats after second week of feeding

Treatment	Latencies							
groups	48°C	49°C	50°C	51°C	52^{0}C	53°C	54°C	55°C
ND	14.8±1.2	17.5±0.6	15.6±0.5	13.1±0.8	10.3±0.8	9.3±1.1	8.1±1.5	6.4±1.3
LP	10.0±1.2^{a}	8.8±0.7^{b}	7.3±0.6^{b}	6.0±0.4^{b}	5.2±0.3^{b}	5.3±0.6^{a}	4.4±0.5^{a}	3.6±0.2^{a}
LPMV	6.3±0.2^{b}	5.6±0.3^{b}	5.1±0.8^{b}	4.1±0.2^{b}	3.1±0.2^{b}	3.0±0.3^{b}	2.7±0.3^{b}	2.0±0.2^{a}
LPV	7.5±0.4^{b}	6.7±0.3^{b}	6.4±0.2^{b}	5.0±0.3^{b}	4.4±0.5^{b}	3.5±0.3^{b}	4.7±0.2^{a}	3.0±0.4^{a}
LPM	6.8±0.3^{b}	5.6±0.2^{b}	5.0±0.2^{b}	4.5±0.3^{b}	4.0±0.1^{b}	4.2±0.1^{b}	4.7±0.2^{a}	2.7±0.2^{a}

ND: Normal kiet, LP: Low protein LPMV: Low protein, Minerals and vitamins, LPV: Low protein and vitamins, LPM: Low protein and minerals. Each value is Mean+SEM of six rats. Significant from the control group (ND) $^{a}p<0.05$, highly significant $^{b}p<0.001$

Table 6: Effects of different diets on tail-flick latency in rats after fourth week of feeding

Treatment	Latencies (sec)							
groups	48°C	49°C	50°C	51°C	52°C	53°C	54°C	55°C
ND	14.8±1.2	17.5±0.6	15.6±0.5	13.1±0.8	10.3±0.8	9.3±1.1	8.1±1.5	6.4±1.3
LP	9.1±0.9^{b}	8.4±0.7^{b}	6.8±0.4^{b}	6.0±0.3^{b}	5.3±0.2^{b}	5.6±0.3^{a}	4.0±0.1^{a}	3.4±0.1^{a}
LPMV	5.0±0.2^{b}	4.3±0.2^{b}	3.7±0.3^{b}	3.1±0.1^{b}	2.5±0.1^{b}	3.3±0.2^{b}	2.4±0.2^{b}	2.5±0.2^{a}
LPV	5.6±0.3^{b}	5.0±0.2^{b}	4.3±0.3^{b}	4.1±0.4^{b}	3.6±0.4^{b}	4.0±0.1^{b}	3.6±0.3^{b}	2.5±0.6^{a}
LPM	5.1±0.2^{b}	4.5±0.2^{b}	4.0±0.1^{b}	4.3±0.3^{b}	3.4±0.1^{b}	3.6±0.2^{b}	3.0±0.2^{b}	1.1±0.5^{b}

ND: Normal diet, LP: Low protein, LPMV: Low protein, minerals and vitamins, LPV: Low protein and vitamins, LPM: Low protein and minerals. Each value is Mean±SEM of six rats. Significant from the control group (ND) $^{a}p<0.05$, highly significant $^{b}p<0.001$

Table 7: Effects of different diets on tail-flick latency in rats after sixth week of feeding

Treatment	Latencies (sec)							
groups	48°C	49°C	50°C	51°C	52°C	53°C	54°C	55°C
ND	14.8±1.2	17.5±0.6	15.6±0.5	13.1±0.8	10.3±0.8	9.3±1.1	8.1±1.5	6.4±1.3
LP	6.6±0.7^{b}	5.6±0.3^{b}	4.3±0.3^{b}	4.0±0.1^{b}	3.5±0.3^{b}	4.0±0.1^{b}	3.2±0.2^{b}	2.9±0.4^{a}
LPMV	3.4±0.2^{b}	2.9±0.2^{b}	2.8±0.2^{b}	2.1±0.2^{b}	1.6±0.2^{b}	1.1±0.1^{b}	1.0±0.5^{b}	1.5±0.6^{b}
LPV	4.8±0.3^{b}	4.3±0.3^{b}	3.5±0.3^{b}	4.0±0.2^{b}	3.8±0.1^{b}	3.3±0.2^{b}	2.7±0.3^{b}	2.2±0.2^{a}
LPM	4.3±0.2^{b}	4.1±0.2^{b}	3.5±0.1^{b}	3.8±0.1^{b}	2.8±0.8^{b}	2.6±0.1^{b}	2.5±0.1^{b}	2.3±0.1^{a}

ND: Normal diet, LP: Low protein; LPMV: Low protein, Minerals and vitamins; LPV: Low protein and vitamins, LPM: Low protein and minerals. Each value is Mean±SEM of six rats. Significant from the control group (ND) $p<0.05^{a}$, highly significant $p<0.001^{b}$

when compared with the normal (ND) fed rats with no significance among the LPMV, LPV and LPM group. LP has the highest latency in the malnourished group.

Formalin Test

The figures show the effect of these different diets for two, four and six weeks respectively on formalin-induced paw licking time. At both Early and Late Phases, a significant increase was observed in the malnourished groups when compared to the control group, $p<0.05$. (Fig. 1a-c). Also, paw licking time was higher in Late Phases compared to the early phase of the test session.

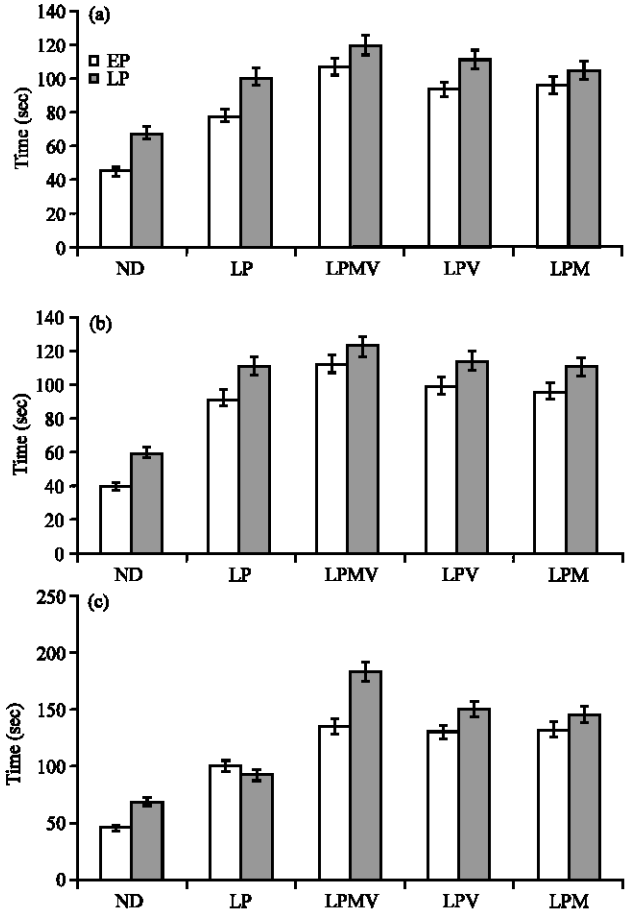

Fig. 1: (a-c) Nociceptive responses in both Early and Late Phases of Formalin Test in Normal and Malnourished animals. The control group (ND) has lowest licking time among the diets, LPMV group has the highest licking time and LP group has the lowest licking time among the malnourished. ND: Normal diet, LP: Low protein diet, LPMV: Low protein, minerals and vitamins Diet, LPV: Low protein and vitamins diet, LPM: Low protein and minerals, EP: Early phase, L P: Late phase. Effect of different diets on formalin induced paw licking test after the (a) second week, (b) fourth week and (c) sixth week of feeding

DISCUSSION

The results of this work follow the same trend for the three different weeks of feeding studied. Rats on normal diet had the highest pain threshold for all the three weeks under study. Rats fed with low protein diet had the next highest latency which was significantly different from the normal diet group. This underscores the importance of protein in pain sensitivity as reported by previous workers. The importance of vitamins and minerals was highlighted by the result obtained in the animals fed with low protein, minerals and vitamins. The animal in this group had the lowest latencies and was significantly lower when compared to the low-protein diet fed animals. The same trend is observed in the formalin-induced paw licking time across the groups.

The tail flick and hot plate tests are based on phasic stimulus of high intensity, the nociception is short lasting and they are thus models acute pain (Tjolsen *et al.*, 1992). Also, the two tests were preferred for this investigation because of their sensitivity and prevention of tissue damage or injury (D'Amour and Smith, 1941). The reduced pain threshold observed in malnourished rats of this study is in agreement with the findings of Rocinholi *et al.* (1997), who demonstrated that protein malnutrition imposed only during the lactation period is efficient in inducing hyper reactivity to electric shock and that environmental stimulation attenuates the differences in shock threshold produced by protein malnutrition. In the thermal tests employed in this study, the degree of decrease of pain threshold depends on the presence or absence of vitamins and minerals supplements. This is manifested in the group of animals fed with low protein, minerals and vitamins which have the lowest pain threshold. It may be due to the absence of the essential nutrients-minerals and vitamins.

Morgane *et al.* (1978) found that the significant effect of protein malnutrition on shock threshold test translate to increase in sensitivity to painful stimuli in lactating animals, as a result of brain deficits and distortions in brain structure produced by protein malnutrition, which causes the impairment of normal brain development of the malnourished animals. Aside the lower body and brain weights in malnourished animals than the control, Almeida *et al.* (1992) demonstrated that protein malnutrition during lactation and post-lactation leads to a lower shock threshold in rats tested during the malnutrition period or later, after nutritional recovery. These and other previously reported results indicate that protein malnutrition causes long-lasting impairment of neuronal system underlying emotional behavior. However, the present study suggests the pivotal role not only protein but vitamins and minerals play on pain perception during malnutrition. In the group of rats fed with low protein diet with mineral supplement only, LPV and low protein diet with vitamin supplement only, LPM, a lower pain threshold was recorded but not as low as in the group fed with low protein without vitamin and mineral supplements, LPMV.

In formalin test, the nociceptive response has two phases. The early acute phase (0-5 min) reflects direct effect of formalin on nociceptive C fibres whereas the late chronic phase (20-40 min) is accompanied by well extended nociceptive response (Hunter and Singh, 1994) and functional changes in nociceptive C fibres (Tjolsen, 1992). Experimental findings have indicated that substance P and bradykinins participate in the early phase while histamine, serotonin and prostaglandins are involved in the late phase (Shibata *et al.*, 1989). The control group has lowest licking time compared with the malnourished, with the LPMV group having the highest licking time among the malnourished. This may imply that malnourished rats have increase sensitivity to painful stimuli.

CONCLUSION

The present study confirms the effects of malnutrition on pain threshold and that the significant potentiation in pain perception is not only associated with protein malnutrition but micronutrient deficiencies. It is interesting to note that supplementing the malnourished animals with proteins, vitamins and minerals increases both pain threshold and weight of animals.

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