

Hepatoprotective Effect of Mineral Supplementation in Early Weaned Rats

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ABSTRACT

Early weaning is becoming increasingly common in developing as well as developed populations. The current study investigated the impact of early weaning and administration of a mineral supplement on the structural integrity of the liver as well as the antioxidant capacity of the liver in a premature weaning model of rats. Twelve Wistar rat pups were divided into normally weaned and early-weaned groups. The normally weaned group (Group A) remained with the dam and were separated from the mothers on the 28th day of postpartum. The early-weaned pups, on the other hand, were separated on the 18th day of postpartum and were subdivided into Group B (fed normal rat chow) and Group C (fed 1 ml/kg of a mineral supplement). On the 35th day of postpartum, all the pups were humanely killed. Body masses, liver ratio, and activity of liver antioxidant enzymes were assessed. Premature weaning had no influence on body masses and organ masses ($p > 0.05$). Liver tissue was normal in all the groups in histological examination. Premature weaning also reduced the levels of markers of oxidative stress, viz., superoxide dismutase (SOD) and glutathione peroxidase (GPx), in the liver of the pups ($p < 0.005$). In the supplemented early-weaned group, SOD and GPx activity was significantly greater in the liver ($p < 0.05$ and $p < 0.5$, respectively). Malondialdehyde (MDA), a marker of lipid peroxidation, was significantly greater in the early-weaned Group B than in the normally weaned Group A and the supplemented early-weaned Group C ($p < 0.005$). Generally, mineral supplementation reduced oxidative damage to the liver as a result of early weaning.

KEYWORDS: Early weaning, Juvenile rats, Hepatic tissue, Oxidative stress, Lipid peroxidation, Mineral supplements, Protective antioxidants.

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INTRODUCTION

Promoting exclusive breastfeeding in the first six months of an infant's life is an important priority in nutritional and public health interventions to reduce child mortality under the age of five years.¹ Exclusive breastfeeding is highly known due to its advantages to infants' and mothers' health. Exclusive breastfeeding is recommended by the World Health Organization (WHO) for the first six months of life, with continued breastfeeding supplemented by the addition of complementary foods at least up to two years for maximum health benefits.² A large percentage of women, however, stop breastfeeding prematurely.³ Breast milk is the optimal diet for infants of all gestational ages.⁴ Made up of 87% water, 1% protein, 4% lipids, and 7% carbohydrates (1–2.4% of which are oligosaccharides), it also has a number of minerals like calcium, phosphorus, magnesium, potassium, and sodium, and vitamins, enzymes, and antibodies.⁵ Breastfeeding has immediate and long-term advantages to infants that include reduced infection and infant mortality rates, improved cognitive and physical development, and protection against obesity and metabolic disease in adult life.⁶

Weaning is defined by the inclusion of complementary foods in an infant's diet, which surpasses the diet received from breast milk alone.⁷ In murine models, this weaning

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process takes approximately 21 to 28 days. Current trends have proven a remarkable decline in breastfeeding rates, especially in urban areas.⁸ Researchers⁹ have reported various factors related to poor breastfeeding practice and complementary feeding strategies through various research. Researchers¹⁰ have noted maternal factors such as age, marital status, work, educational level, access to maternal and neonatal care, exposure to information about health, socioeconomic status, cultural practices, and location, along with infant-related factors such as birth weight and method of delivery. Early weaning is stressful to the infant and causes impairment of growth. This practice inhibits the activity of gut enzymes and triggers protein degradation, which negatively affects the immune system, increases cytokines, and activates signalling pathways.¹¹ In addition, weaning interferes with the balance of redox processes, leading to oxidative stress and apoptosis of cells.¹²

Another study demonstrated that the activity of antioxidant enzymes has been regarded as the most important marker of hepatic function in young, weaned pigs.¹³

Even though it has a role, weaning and its role in liver health have not been studied extensively. The liver is the major site of metabolic processes in which there is a dense population of mitochondria responsible for the production of reactive oxygen species.¹⁴ In our previous report,¹⁵ weaning was found to induce oxidative stress in the livers of pigs, inducing apoptosis of cells via the MAPK signaling pathway. Antioxidant enzymes are important in the regulation of hepatic metabolism, the role of which is of critical importance in liver disorders.¹⁶ Oxidative stress caused by reactive oxygen species (ROS) can cause deleterious effects on proteins, nucleic acids, and cell membranes, thus compromising immune responses and defense mechanisms in weaning animals.¹⁷ Catalase, superoxidodismutase, and glutathione peroxidase enzymes are involved in reducing oxidative stress.¹⁸

Micronutrient deficiencies in infant formulas have adverse effects on newborns and are correlated with high infection and disease susceptibility rates.¹⁹ On the other hand, overabsorption of micronutrients may also result in health disorders.²⁰ Malnutrition during this pivotal development phase can hinder brain development and organ function. High magnesium absorption has been correlated with reduced prevalence of fatty liver disease and prediabetic conditions. Numerous research studies have been carried out to investigate the role of magnesium that serves as a cofactor in more than 300 metabolic functions of human physiology.²² Magnesium plays a crucial role in several physiological functions such as DNA and protein synthesis, glucose and lipid metabolism, and oxidative phosphorylation.²³ Magnesium deficiency has been shown to induce inflammatory reactions, mitochondrial dysfunction, fibrotic mechanisms, and reduced antioxidant activity, resulting in lipid peroxidation, cytokine activation, steatohepatitis, and scarring of the liver. By 2010, only one controlled trial was published on the effect of magnesium supplementation on ALT levels.²⁴ Calcium absorption in infants is largely passive and is mediated by the lactose in human milk. With advancing age, passive absorption declines and active calcium absorption in the intestine stimulated by calcitriol becomes more pronounced. Maternal calcium intake does not seem to influence regulation of calcium in human milk.²⁵

During the first six months of nursing, human breast milk usually gives an infant two to three times the amount of calcium that the fetus would gain from the mother in the full nine months of pregnancy. This experiment tested the influence of early weaning and mineral supplementation on the cellular architecture and antioxidant activity of young rat livers.

MATERIALS AND METHODS

Animals Utilized in Experiments and Their Controls

This investigation was conducted in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin. Internationally accepted procedures were maintained during the treatment of the animals. Forty-eight rats (48; 36 females and 12 males), weighing between 100 g and 120 g, were obtained from Bankylatt Ventures, Ogbomoso, Oyo State, Nigeria. The rats were fed rat chow from Ogo-Oluwa Feeds and Four Mill Limited, Sango, Ilorin, Kwara State. The animals were given a 14-day adaptation period before the experiment, during which the masses were measured on a regular basis using a sensitive scale. After the adaptation period, the animals were separated into three females and one male. Female rats were weighed daily and monitored for signs of pregnancy. After giving birth, each dam and pups were housed in separate cages to prevent any losses due to overcrowding.

Animal Classification and Supplement Use

Day of delivery was considered day zero. Twelve (12) pups were separated into three groups based on weaning time: Group A, as positive control, had pups separated on the 28th day of birth; Group B, as negative control, had pups separated on the 18th day of birth and given normal rat chow; and Group C had pups that were given a vitamin B-complex supplement (given at 1 ml/kg body weight) after separation on the 18th postnatal day. This supplement was given orally via a tube for 17 days. Body weight (in grams) of each pup was recorded every three days, starting from the date of birth to the 35th day of birth.

Specimen Collection and Animal Euthanasia On the 35th day of birth, pups were euthanized humanely. For histochemical examination, pups were sedated with 0.5 ml of ketamine HCl prior to proceeding. Following sedation, the chest cavity of each rat was opened, and a syringe with normal saline was injected into the right ventricle of the heart to flush the system. Subsequently, 4% formaldehyde was flushed into the ventricular system in the same manner as the saline to fix the organ tissue. The liver was then quickly removed and held in a paraformaldehyde solution. The tissues were stained with hematoxylin, and eosin (H&E) for microscopic examination. Rat pups to be used for biochemical examination were euthanized via cervical dislocation. They were opened, their livers removed, weighed, and placed in containers with a 30% sucrose solution pre-cooled on ice and kept in a cold environment. The samples were then later homogenized into a homogeneous mixture and centrifuged at 3000 rpm.

Statistical Analysis

The results of the experiments are reported as the mean \pm SEM. All the data were analyzed by GraphPad Prism

Table 1: displaying average starting and ending body weight, liver weight, and proportional liver weight in rats from Control Group A and Experimental Groups B and C.

| Groups of rats | Average IBW (g) | Average FBW (g) | % WT change (g) | Average Liver Weight (g) | RLW |
|----------------|-----------------|-----------------|-----------------|--------------------------|-------|
| B A | 5.37 ± 0.05 | 37 ± 0.5 | 15% | 0.25 ± 0.05 | 0.008 |
| B | 5.10 ± 0.75 | 41 | 12% | 0.20 ± 0.01 | 0.006 |
| C | 5.02 ± 0.05 | 37 ± 0.25 | 14% | 0.20 ± 0.02 | 0.006 |

Table 2: Illustrates the average and standard deviation of the body mass.

| | A | B | C |
|--------------------|-------|-------|--------|
| Mean | 34.88 | 34.39 | 32.23 |
| Standard deviation | 2.551 | 1.693 | 0.6500 |

software (version 5.0, La Jolla, CA). The morphological and biochemical findings were evaluated by one-way analysis of variance (ANOVA), along with Tukey's post hoc analysis. Comparison between multiple groups was conducted, and $p < 0.05$ was considered to be statistically significant.

RESULTS

This research assiduously documented the morphological, histological, and biochemical impacts of early weaning and mineral supplementation on liver tissue. All the relevant data and findings were documented to accuracy.

Observations

The feeding and excretion habits of the weaned rats on day 28 of birth were normal. These rats gained weight significantly, were non-aggressive, had adequate resting periods, and had plenty of energy. Also, in contrast to the mature mothers of the prematurely weaned rats, no aggressive behaviors were displayed by the mothers of the other two groups. The prematurely weaned groups' offspring had poor food consumption, recorded high weight loss after weaning that took a long time, and had low activity.

Observations about Morphology the final body weight of the animals in Control Group A and Experimental Groups B and C was greater compared to their initial body weight. No differences were seen in the masses of the livers or their relative proportion between the groups (Table 1).

Organ Weight

The masses and proportional masses of the liver displayed no substantial differences among the groups (Table 1).

Histological Observation

Typical micrographs of the livers from all rat groups reveal a standard hepatic arrangement, with the central vein clearly visible (Figure 4). There are no halo areas or bile accumulations present.

Biochemical Examination

In this research, the activities of MDA, SOD, and GPx were evaluated, and compared to groups A and C, animals in group B showed a notable ($p < 0.005$) reduction in GPx concentrations. In this investigation, the delivery of vitamin B-complex halted lipid peroxidation because the premature weaning group demonstrated heightened ($p < 0.005$) MDA levels relative to the other treatment categories.

Superoxide Dismutase (SOD)

SOD concentrations were substantially elevated in the group treated with vitamin B-complex, group C, in contrast to groups A and B, and statistically reduced in group B relative to group A. Heightened SOD levels in rats receiving mineral supplementation were observed to guard against degenerative modifications.

Malondialdehyde

The MDA concentrations in the premature weaned group B were markedly higher than in groups A and C. However, no notable difference existed between the MDA concentrations of group A and C pups.

Glutathione Peroxidase

The GPx concentration in group B was reduced compared to groups A and C. This variance was notable relative to group A but lacked significance relative to group C.

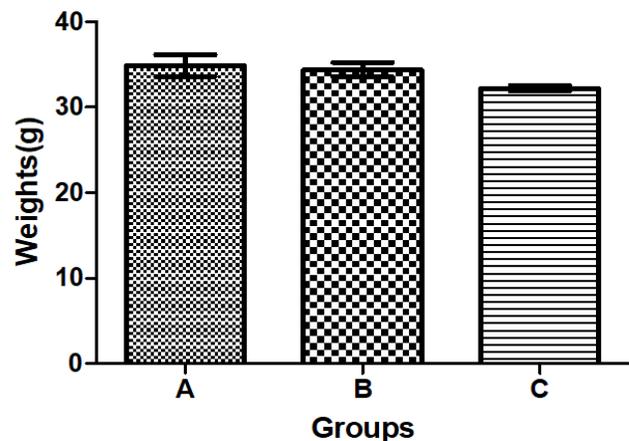


Figure 1: Illustrates the body mass growth among rat groups ($p > 0.05$) (Group A = standard weaned group, Group B = premature weaned group, Group C = Premature weaned + Vitamin B-complex group).

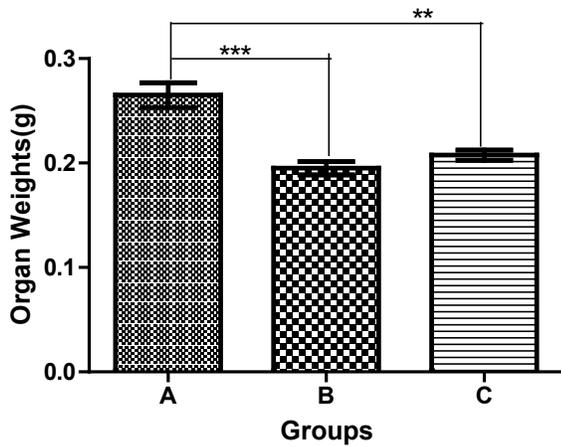


Figure 2: Depicts a rise in organ mass among rat groups. $p < 0.05$. *** and ** indicate significant thresholds of $p < 0.005$ and $p < 0.01$, respectively. ($p = 0.0005$).

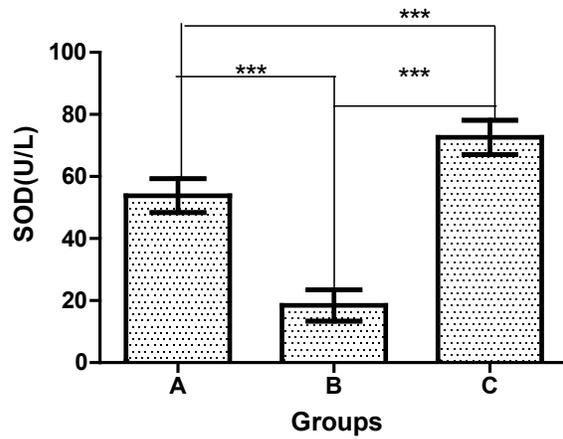


Figure 5: Hepatic level of superoxide dismutase. A normal weaned, B early weaned, C early weaned + Vitamin B-complex supplement. *** represents a significance level of $p < 0.005$. ($p = < 0.0001$)

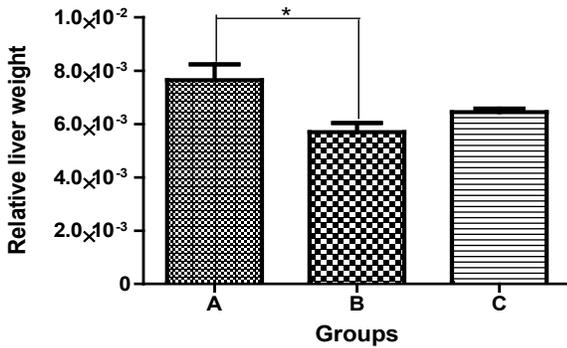


Figure 3: Shows the relative liver weight between rat groups. * represents a significant level of $p < 0.05$. ($p = 0.0209$).

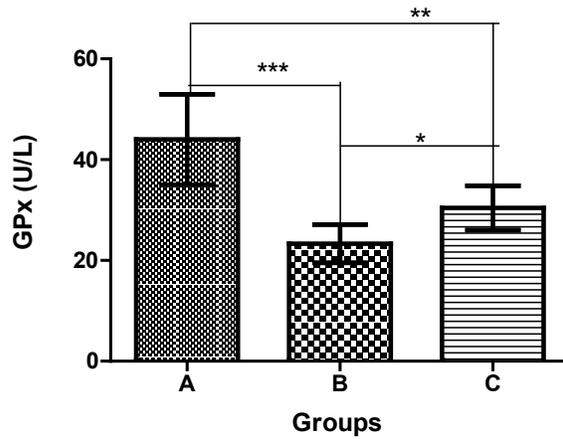


Figure 7: Hepatic levels of Glutathione Peroxidase. A= Normal-weaned, B= Early-weaned, C= Early-weaned+ Vitamin B-complex supplement. ***, ** and * represents significance levels of $p < 0.005$, $p < 0.001$ and $p < 0.5$ respectively. ($p = 0.0001$).

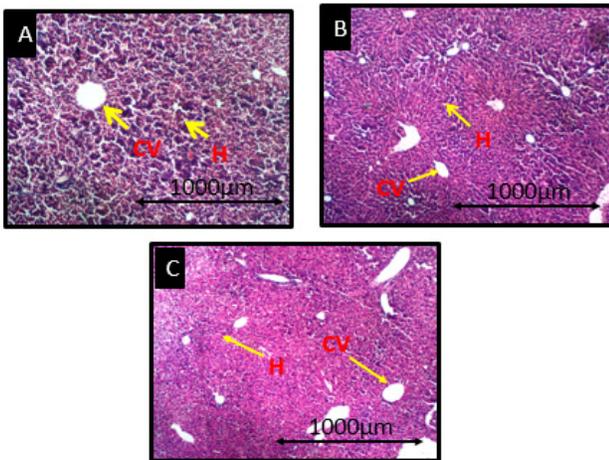


Figure 4: Representative micrographs of the liver of Wistar rats showing the central vein (CV) and the surrounding hepatocyte (H). (A = Early-weaned group, B= Normal-weaned group, C = Early-weaned + mineral supplement group)

DISCUSSION

Control of body mass relies primarily on changes in cellular power expenditure. Therefore, increases in power demands are counterproductive to control of a constant body mass. Mass loss was anticipated with diet change and decreased nutrient consumption because of early weaning; nevertheless, the animals rapidly replaced the lost mass. The group supplemented with minerals lost mass soon after weaning because of insufficient initial adaptation and feeding behavior. Nevertheless, after acclimatization to the new diet regimen and environment, the rats quickly regained the lost mass. The gains in mass in the normal-weaned rats were consistent for a while before gradually increasing. Hence, there were no critical mass differences among the rat groups. Rats initiate the intake of solid food towards the end of lactation but still consume milk throughout terminal weaning.

The initial mass loss may be attributed to the abrupt shift from the intake of maternal milk to the intake of solid foods of varied nutritional composition.

Oliveira *et al.*, (2011) investigated the effect of weaning on satiety-related behavior and feeding patterns in adult rats. Weaning of rat pups was performed on postnatal days 15, 21, and 30. Manipulation of weaning day did not produce differences in body mass.²⁶ This is in line with the findings of the present study. Other studies indicated that early weaning resulted in higher body mass by suppressing lactation by maternal bromo ergocriptine treatment.²⁷⁻²⁹ The structural features of the liver in the three animals were normal and had no histological variations. It therefore means that normal weaning, premature weaning, and mineral supplementation cannot affect the natural histological structure of the liver.

For this study, malondialdehyde (MDA) was used to determine oxidative status. Antioxidant capacity, as a measure of superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels, was also used. Our findings reported increased levels of oxidation. Oxidative damage-induced cell, tissue, and organ damage is due to overoxidation. Increased levels of free radicals, or reactive oxygen species (ROS), certainly lead to direct lipid damage.³⁰ Lipid peroxidation, or free radical oxidation of polyunsaturated fatty acids in biological systems, is of critical importance to comprehend. Furthermore, the identification and quantitation of lipid peroxidation give the most conclusive evidence for the involvement of free radical mechanisms in toxicity.

Malondialdehyde is one of the products of lipid peroxidation and is used as an indicator in the estimation of oxidative stress.³¹ Estimation of malondialdehyde gives valuable information regarding the degree of oxidative damage, which is found to be crucial in the understanding of various pathological conditions.^{31,32} Here, premature weaning stimulated lipid peroxidation, as reflected by increased levels of MDA ($p < 0.05$) in the prematurely weaned group compared with the other treatment groups. The ability of mineral supplements to suppress the levels of MDA after lipid peroxidation induced by premature weaning is due to their antioxidant activity and free radical scavenging activity.

The research revealed that glutathione peroxidase (GPx) levels were significantly greater in the control weaned group compared to the prematurely weaned group and the mineral-supplemented group. The finding indicates the significance of weaning compared to GPx levels. High GPx levels indicate a significant internal antioxidant defense system within the brain that is engaged in the removal of hydrogen peroxides and lipid peroxides. The GPx level recorded in the prematurely weaned group was significantly lower ($p < 0.05$) compared to the mineral supplement group.

Such a finding may be attributed to the free radical scavenging capability and antioxidant activity of mineral supplementation.

Concurrently, the findings of this experiment affirm that the activity level of superoxide dismutase (SOD) significantly rose in the control and mineral supplement groups and significantly fell in the premature weaning group. This remarkable rise in the activity level of SOD in the mineral supplement group lends credence to our belief that mineral supplements are antioxidative in nature and yield free radical scavenging activity.

CONCLUSION

The results of the study show that a vitamin B complex mineral supplement did not affect the cellular content of the livers of Wistar rats. It was, however, noted to decrease the cellular oxidation levels involved in the weaning process by virtue of its antioxidant nature. Weaning is a critical phase that is increasingly taking place at younger ages in the modern world due to various reasons such as occupational demands and maternal health problems. It is therefore critical to determine the correct nutritional replacements for infants after weaning to ensure proper growth and development.

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