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Enhanced Lymphatic Absorption of Vitamin D3 Delivered in Mealworm (Tenebrio molitor) Oil Compared to Lichen- and Lanolin-Based Commercial Oils in a Rat Model

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Abstract

Background: The intestinal absorption of vitamin D3 (cholecalciferol) is strongly influenced by the lipid matrix in which it is delivered. Insect-derived oils have emerged as novel nutritional lipid sources and may enhance the bioavailability of fat-soluble vitamins.

Objective: To compare the lymphatic absorption of vitamin D3 delivered via Tenebrio molitor (mealworm) oil with two commercial oils enriched with vitamin D3 from lichen or lanolin.

Methods: Adult male Wistar rats underwent thoracic lymph duct cannulation and received gastric intubation with: (1) vitamin D3–rich mealworm oil (TM-A), (2) lower-dose mealworm oil (TM-E), (3) vitamin D3 from lichen in rapeseed oil, or (4) vitamin D3 from lanolin in rapeseed oil. Lymph was collected for 6 h and analyzed by HPLC for cholecalciferol and by GC for fatty acid content.

Results: Absorption rates ranged from 13–22%. TM-A (21.8%) and lichen oil (19.4%) showed significantly higher vitamin D3 absorption than lanolin oil (13.1%). TM-A produced 2–3-fold higher lymphatic enrichment of vitamin D3 compared with reference oils. TM-E (half-dose) produced lymphatic concentrations comparable to full-dose lichen and lanolin oils. Mealworm oil showed 100% fatty acid absorption and significantly higher lymph lipid content versus reference oils.

Conclusion: Mealworm oil markedly enhances vitamin D3 absorption compared to lichen and lanolin carriers. Its MUFA-rich matrix appears to optimize intestinal uptake, suggesting mealworm oil as a promising nutraceutical delivery system for fat-soluble vitamins.

Keywords: vitamin D3, bioavailability, lymphatic absorption, Tenebrio molitor, insect oil, fatty acids, nutraceuticals

1. Introduction

Vitamin D_3 plays a multifaceted physiological role that extends far beyond calcium and bone homeostasis, encompassing immune modulation, endocrine regulation, cardiometabolic signaling, and cellular differentiation. Recent epidemiological evidence links suboptimal serum 25(OH) D levels with increased risk of autoimmune disorders, metabolic syndrome, infectious diseases, and impaired musculoskeletal performance. Consequently, improving the bioavailability of dietary vitamin D_3 has become an important public health objective. However, the extent to which vitamin D_3 is absorbed and utilized is contingent upon the lipid matrix through which it is delivered, as micellarization, enterocyte uptake, and chylomicron secretion are highly dependent on fatty acid composition and matrix polarity.

Lipid structural features, including chain length, degree of unsaturation, and stereochemical orientation, have been shown to influence the kinetics of intestinal solubilization and subsequent lymphatic transport of fat-soluble nutrients. Research indicates that MUFA-rich matrices tend to facilitate micelle formation and absorption, whereas certain PUFA-dominant oils may exert competitive effects on lipid transport pathways. Against this biochemical backdrop, edible insect oils have emerged as novel, sustainable lipid sources containing unique fatty acid profiles capable of enhancing nutrient delivery.

Mealworm (Tenebrio molitor) oil, in particular, is characterized by a favorable MUFA-to-SFA ratio and bioactive lipid fractions that may synergistically enhance micellar incorporation of cholecalciferol. Yet, despite rising interest in entomological oils as functional carriers, limited empirical evidence exists regarding their capacity to modulate intestinal absorption of vitamin D_3. Accordingly, the present study evaluates the lymphatic bioavailability of vitamin D_3 delivered in mealworm oil relative to two commercial matrices derived from lichen and lanolin, using a lymph-cannulated rat model - a gold-standard method for quantifying lipid transport.

2. Materials and Methods

2.1 Study design

An established lymph-cannulated Wistar rat model was used to enable continuous, temporally resolved collection of postprandial lymph following administration of vitamin D_3-enriched oils. This model is widely recognized as the most physiologically accurate system for characterizing lipid-phase nutrient absorption because it captures chylomicron-mediated transport prior to hepatic first-pass metabolism.

Oils were fully characterized by HPLC-DAD for vitamin D_3 concentration and by GC-FID for fatty acid composition to ensure mechanistic interpretation of absorption differences. Animals underwent a standardized fasting protocol to normalize intestinal transit and received a single intragastric dose calibrated to deliver either 50 µg or 25 µg vitamin D_3 depending on group assignment. Lymph was collected continuously for 6 h into chilled, EDTA-treated tubes to prevent lipid oxidation.

Vitamin D_3 quantification followed validated extraction workflows employing saponification, non-polar phase separation, and spectrally verified chromatographic detection. Fatty acid analysis used reference standards and calibration curves to ensure accuracy. The absorption coefficient (T) was computed using tracer-normalized reference values to yield quantitative estimates of intestinal bioavailability. Statistical comparisons employed ANOVA followed by Tukey's test, with significance defined as $p < 0.05$.

2.2 Test oils

Four formulations were evaluated: (1) TM-A, a mealworm oil enriched to 24.6 µg/mL vitamin D3; (2) TM-E, a mealworm oil with 11.2 µg/mL vitamin D3; (3) a lichen-derived vitamin D3 formulation diluted in virgin rapeseed oil to 28.6 µg/mL; and (4) a lanolin-derived vitamin D3 formulation diluted in virgin rapeseed oil to 23.5 µg/mL. A control group received physiological saline. The fatty acid compositions of the oils included MUFA, PUFA (n-6 and n-3), and saturated fatty acids (SFA).

Table 1. Vitamin D3 content and fatty acid composition of the test oils.

Oil	Vit D3 (µg/mL)	MUFA (%)	PUFA n-6 (%)	PUFA n-3 (%)	SFA (%)
TM-A	24.6	42.7	28.0	1.4	27.6
TM-E	11.2	58.6	20.6	9.7	11.0
Lichen-D3	28.6	58.5	20.7	9.7	11.1
Lanolin-D3	23.5	59.2	20.8	10.1	10.7

2.3 Dosing and administration

All treatment groups received an oil volume calibrated to deliver 50 µg vitamin D3, except the TM-E group, which received 25 µg vitamin D3 to explore a potential dose–response effect. Oils were administered once by gastric intubation following a fasting period.

2.4 Lymph collection and analysis

Thoracic lymph was collected continuously for 6 h after intubation. Vitamin D3 (cholecalciferol) concentrations in lymph were quantified using high-performance liquid chromatography equipped with diode-array detection. Fatty acid profiles were determined by gas chromatography with flame ionization detection.

2.5 Calculation of absorption rate

The intestinal absorption rate (T) of vitamin D3 was expressed relative to a lipid tracer standard assumed to have 100% absorption, according to the equation:

$$T = (RL / RH) \times 100$$

where RL is the amount of vitamin D3 recovered in lymph relative to the reference and RH is the amount of vitamin D3 ingested relative to the reference dosage.

3. Results

3.1 Lymphatic vitamin D3 concentrations and absorption

Lymphatic vitamin D3 concentrations and calculated absorption rates are summarized in Table 2. Mealworm oil formulations (TM-A and TM-E) showed higher absorption rates than the lanolin-based formulation. TM-A produced 2–3-fold higher lymphatic enrichment compared with the commercial reference oils, despite equivalent dosing.

Table 2. Lymphatic vitamin D3 and absorption rate for each group.

Group	Vit D3 lymph (arbitrary units)	Absorption (%)
TM-E	21.7 ± 8.1	21.7
TM-A	21.8 ± 1.3	21.8
Lichen-D3	19.4 ± 2.8	19.4
Lanolin-D3	13.1 ± 3.6	13.1
Control	ND	—

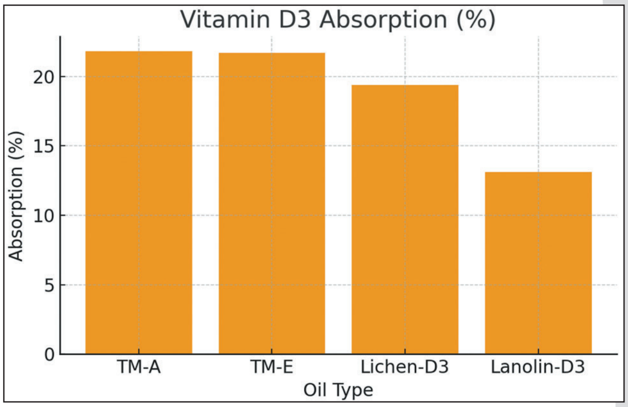

Figure 1. Vitamin D3 Absorption by Oil Type

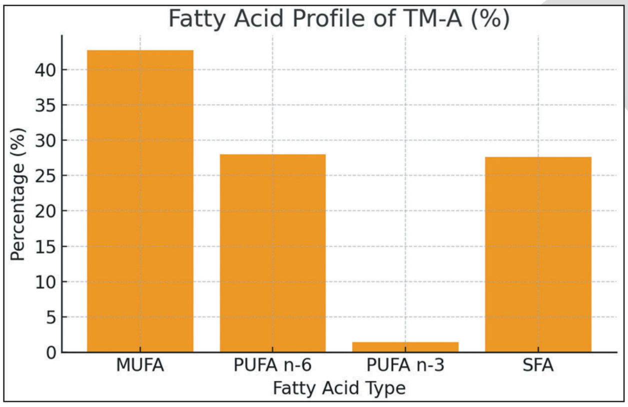

Figure 2. Fatty Acid Composition of TM-A Oil

3.2 Lymphatic fatty acid absorption

Total fatty acid absorption from mealworm oils reached 100%, as reflected by the high concentration of lymphatic fatty acids in TM-A and TM-E groups compared to lichen- and lanolin-based formulations. The lymphatic fatty acid profile closely mirrored the composition of the ingested oils.

4. Discussion

The findings of this study reveal a pronounced enhancement of vitamin D_3 absorption when delivered through mealworm oil matrices, with TM-A demonstrating the highest bioefficacy. From a mechanistic perspective, the MUFA-enriched lipid environment likely improves micelle fluidity, reduces steric interference during solubilization, and enhances substrate affinity for enterocyte transporters, including SR-B1 and NPC1L1. Moreover, the comparatively lower n-3 PUFA content of mealworm oil may reduce competitive interference during microsomal triacylglycerol resynthesis—one of the rate-limiting steps in chylomicron assembly. The observation that TM-E, despite its substantially reduced vitamin D_3 content, achieved lymphatic concentrations similar to the commercial lichen and lanolin oils indicates that matrix-driven enhancements can compensate for dose reductions. This aligns with studies demonstrating that nutrient–lipid interactions, rather than nutrient mass alone, can govern absorption kinetics. Furthermore, the complete fatty acid recovery from the mealworm treatments supports the notion that entomological lipids undergo efficient enzymatic hydrolysis and assimilation, consistent with emerging research on their digestive compatibility. These results collectively strengthen the rationale for incorporating mealworm oil into fortified food systems aimed at improving global vitamin D nutritional status. Future work should include structured lipidomics profiling, human pharmacokinetic trials, and exploration of synergistic formulations combining mealworm oil with emulsifiers or bioactive phospholipids to further potentiate vitamin D absorption.

5. Conclusion

Mealworm (Tenebrio molitor) oil significantly enhances the lymphatic absorption of vitamin D3 relative to lichen- and lanolin-based oils in a lymph-cannulated rat model. Its lipid profile appears to optimize intestinal uptake, making it a promising delivery platform for fat-soluble vitamins. Further clinical studies in humans are warranted to confirm these preclinical observations and to explore applications in fortified foods and dietary supplements.

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Determination of lactate dehydrogenase in pregnancy and fetus with preeclamsia is an important parameter of fetus growth retardation

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1. Abstract

Introduction - It is known that in preeclampsia, especially in the placenta, atherosclerosis of blood vessels develops and exists. Due to damage to the blood vessels, the accumulation of amorphous material and the deposition of immunoglobulins G (IgG), immunoglobulin M (IgM) and activation of the complement, eventually obliteration of the blood vessels develops and ischemia and infarction of the placenta follows.

Recent studies have shown a close relationship between the concentration of lactate dehydrogenase and growth retardation in utero in preeclampsia (26.27). There is a correlation between the level of estriol, placental perfusion, lactate dehydrogenase and fetal growth retardation in utero (28). 5 types of lactate dehydrogenase isozymes have been detected in the placental tissue. LDH_4 and LDH_5 isoenzymes have been found in high concentrations in the placenta (29).

Some studies show that the liver of the fetus is sensitive to hypoxia, as a result of stagnation in the growth of the liver and degeneration of the tissues in the absence of the liver. These scientists have argued that the fetal liver is more sensitive to growth retardation compared to the circumference of the head and abdomen (36, 37, 38).

Aminotransferases, lactate-dehydrogenase, bilirubin (unconjugated, conjugated) are metabolites that change their concentrations depending on liver hypoxia. These scientists have found that it is important to determine between the level of aminotransferases (AST, ALT), lactate dehydrogenase, bilirubin (unconjugated and conjugated) in the blood of the fetus/newborn with growth retardation due to hypoxia (40, 41, 42, 43, 44).

The aim of the paper: The purpose of the paper is to determine and analyze the level of lactate dehydrogenase in the blood of pregnant women with normal and preeclamptic pregnancy as well as in the blood of the fetus in pregnant women with normal and preeclamptic pregnancy in predicting fetal growth retardation.

Material and methods :120 pregnant women were included in the study, of which 60 pregnant women are with physiological pregnancy and 60 pregnant women are with preeclampsia and IUGR.

In pregnant women with physiological and preeclamptic pregnancies, blood is taken from the cubital vein, while in fetuses/neonates with physiological and preeclamptic pregnancies, blood is taken from the umbilical vein to determine the laboratory parameters: lactate dehydrogenase and cholesterol.

In fetuses with physiological and preeclamptic pregnancy, ultrasonographic parameters are determined: biparietal diameter (BPD), femur length (FL). The selection of the group of pregnant women with preeclampsia is formed based on the determination of the method of the last menstruation, Hadlolck's formula, based on the presence of proteinuria (>0.5 g/l) as well as high blood pressure above TA = 140/ 90 mmHg.

Analysis of variables was performed using Pearson's x^2 test or Fisher's exact probability test.

Analysis of normally distributed continuous ratio features was performed using the independent simple t-test, for independent samples, while nonparametrically distributed numerical variables were analyzed using the Mann-Whitney U test for independent samples. Pearson's and Spearman's correlation coefficient was used according to the type of normality and distribution data.

Research results - With the analysis of the Mann Whitney - U test, we found that there is a statistically significant difference in the AST values in serum between pregnant women with physiological and preeclamptic pregnancy [U=521.500, z=6.717, p<0.001]. Preeclamptic pregnancies had higher serum AST values [Me=27.00 U/L (IQR=22.00 to 34.00)] compared to physiological pregnancies [Me=14.00 U/L (IQR=11.00 to 19.00)].

Analysis by means of the Mann Whitney - U test has found that there is a statistically significant difference in serum LDH values between fetuses with stunted growth and fetuses with normal development and growth [U=1203.000, z=-3.135, p=0.002]. Growth retardation (IUGR) fetuses have higher serum LDH values [Me=795.00 U/L (IQR=491.00 to 1490.00)] compared to fetuses with normal growth and development [Me=587.00U/L (IQR =376.00 to 783.00)].

Our study with the help of the correlation coefficient according to Sperman has revealed that there is no significant difference in the linear correlation between the week of gestation and LDH values in the serum of the fetus/newborn with normal growth and development (r_s=0.242; p>0.05).

Our study by means of the correlation coefficient according to Spearman has found that there is a statistically significant difference of the positive linear correlation between the week of gestation and LDH values of the fetus/newborn with growth retardation IUGR (rs=0.274; p> 0.05).

Discussion - The increase in lactate dehydrogenase values is explained by the action of oxidative stress and the disorder of the synthesis of nitrogen monoxide, prostacyclin, coagulation factors, endothelin components, neuropeptides (10) which cause degenerative changes in hepatocytes with an increase in the level of lactate dehydrogenase.

In fetal growth retardation due to hypoxia, which develops into preeclampsia, the activity of lactate dehydrogenase increases, which affects the glycolytic process of carbohydrate breakdown. In the conditions of hypoxia, damage to the liver cells follows, as well as the disorder of the permeability of the cell membrane, in some cases, the transfer of lactate dehydrogenase from the cell to the serum follows, as well as the increase in the level in the blood.

The other mechanism is explained by the fact that the increase in the level of LDH follows due to stress and hypoxia of the fetus as well as due to the increase in fetal cortisol from the adrenal gland. It is assumed that the high level of fetal prolactin stimulates the synthesis of lactate dehydrogenase in the fetal liver.

Conclusion - On the basis of the purpose of the research, the material of the methods and the results, we found these conclusions.

Preeclamptic pregnancies had higher serum LDH values [Me=509.50 U/L (IQR=297.00 to 796.25)] compared to physiological pregnancies [Me=356.50 U/L (IQR=296.50 to 456.00)].

Growth retardation (IUGR) fetuses have higher serum LDH values [Me=795.00 U/L (IQR=491.00 to 1490.00)] compared to fetuses with normal growth and development [Me=587.00U/L (IQR =376.00 to 783.00)].

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Our study by means of the correlation coefficient according to Spearman has found that there is a statistically significant difference of the positive linear correlation between the week of gestation and LDH values of the fetus/newborn with growth retardation IUGR (rs=0.274; p> 0.05).

Key words - Lactate dehydrogenase, Pregnancy, Fetus, Preeclampsia, IUGR.

2. Introduction

It is known that in preeclampsia, especially in the placenta, atherosclerosis of blood vessels develops and exists. Due to damage to the blood vessels, the accumulation of amorphous material and the deposition of immunoglobulins G (IgG), immunoglobulin M (IgM) and activation of the complement, eventually obliteration of the blood vessels develops and ischemia and infarction of the placenta follows.

Recent studies have shown a close relationship between the concentration of lactate dehydrogenase and growth retardation in utero in preeclampsia (26.27). There is a correlation between the level of estriol, placental perfusion, lactate dehydrogenase and fetal growth retardation in utero (28). 5 types of lactate dehydrogenase isozymes have been detected in the placental tissue. LDH_4 and LDH_5 isoenzymes have been found in high concentrations in the placenta (29).

Some studies show that the liver of the fetus is sensitive to hypoxia, as a result of stagnation in the growth of the liver and degeneration of the tissues in the absence of the liver. These scientists have argued that the fetal liver is more sensitive to growth retardation compared to the circumference of the head and abdomen (36, 37, 38).

In fetal growth retardation, changes in the fetal ECG have also been recorded, which are manifested by an elevation of the T-wave (39). It is known that these biochemical parameters appear and are released in the blood before the degenerative morphological changes in the cells.

Aminotransferases, lactate-dehydrogenase, bilirubin (unconjugated, conjugated) are metabolites that change their concentrations depending on liver hypoxia. These scientists have found that it is important to determine between the level of aminotransferases (AST, ALT), lactate dehydrogenase, bilirubin (unconjugated and conjugated) in the blood of the fetus/newborn with growth retardation due to hypoxia (40, 41, 42, 43, 44).

The aim of the paper - The purpose of the paper is to determine and analyze the level of lactate dehydrogenase in the blood of pregnant women with normal and preeclamptic pregnancy as well as in the blood of the fetus in pregnant women with normal and preeclamptic pregnancy in predicting fetal growth retardation.

Material and methods - 120 pregnant women were included in the study, of which 60 pregnant women are with physiological pregnancy and 60 pregnant women are with preeclampsia and IUGR.

In the study, the group of pregnant women with physiological pregnancy included pregnant women whose pregnancy developed in a physiological way with the birth of a child with a normal weight corresponding to the gestational age, normal development of the placenta, umbilical cord and amniotic membranes.

In pregnant women with physiological and preeclamptic pregnancies, blood is taken from the cubital vein, while in fetuses/neonates with physiological and preeclamptic pregnancies, blood is taken from the umbilical vein to determine the laboratory parameters: lactate dehydrogenase and cholesterol.

In fetuses with physiological and preeclamptic pregnancy, ultrasonographic parameters are determined: biparietal diameter (BPD), femur length (FL). The selection of the group of pregnant women with preeclampsia is formed based on the determination of the method of the last menstruation, Hadlolck's formula, based on the presence of proteinuria (>0.5 g/l) as well as high blood pressure above TA = 140/ 90 mmHg.

Pregnant women with diabetes mellitus, anemia in pregnancy, lupus erythematosus, autoimmune diseases, hepatitis (A, B, C, D and E), pregnant women with cardiovascular disease, kidney disease and pregnant women with fetal abnormalities were excluded from the study.

3. Ultrasonographic research methods

Measurement of biparietal diameter (BP) by ultrasonography.

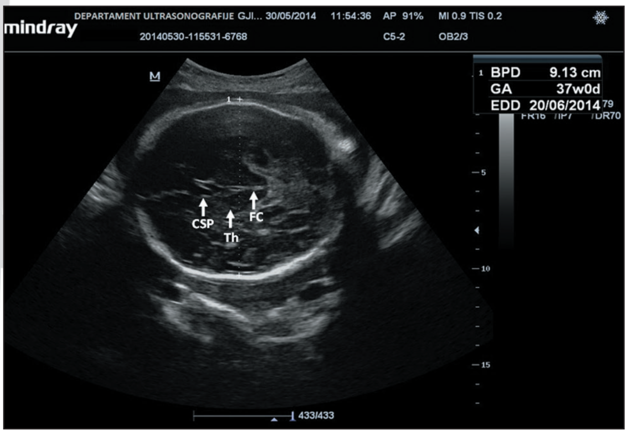


Figure 1. Measurement of biparietal diameter and showing the plan for ultrasonographic measurement of biparietal diameter. Cavum septum pellucidum (CSP), Thalamus (Th), Falcs cerebri (FC).

Femur length measurement by ultrasonography.

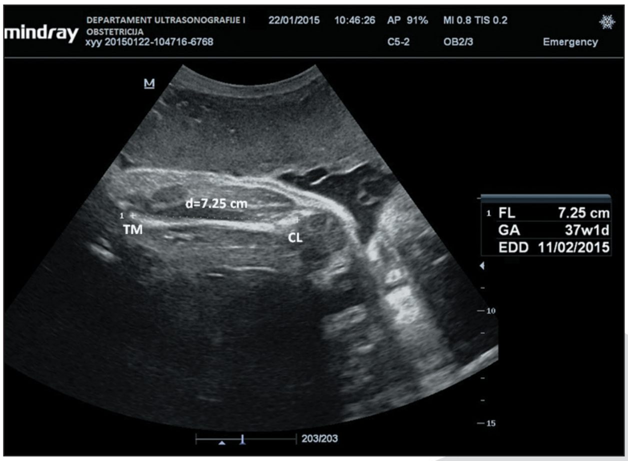


Figure 2. Presentation of femur length measurements. The femur is measured in the length of the ossified part of the femoral diaphysis in the longitudinal section. The femur is measured from the major trochanter to the lateral Condylus. Major trochanter (TM). Condylus lateralis (CL).

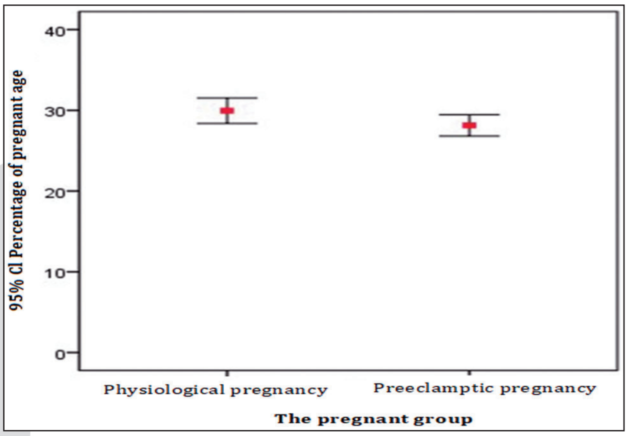
Examination of the normality of the distribution of continuous numerical changes was performed by inspection of histograms, quantile plots, and formal testing using the Kolgomorov-Smirnov test. Analysis of variables was performed using Pearson's x^2 test or Fisher's exact probability test. Analysis of normally distributed continuous ratio features was performed using the independent simple t-test, for independent samples, while non-parametrically distributed numerical variables were analyzed using the Mann-Whitney U test for independent samples. Pearson's and Spearman's correlation coefficient was used according to the type of normality and distribution data. The threshold of statistical significance was set at the conventional level of α =0.05.

4. Research results

4.1. Demographic and laboratory data of the research

1. Age of pregnant women

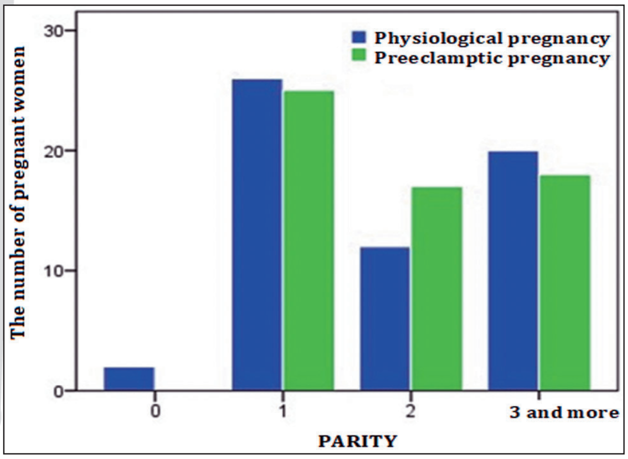
Pregnant women with physiological pregnancy were older [=29.95, SD=6.10; 95% CI (28.37, 31.53)] compared to pregnant women with preeclamptic pregnancy [=28.13, SD=5.10; 95% CI (26.82; 29.45)]. Specified age difference [1,817; 95%CI (-0.216; 3.849)] is not statistically significant [t (118) = 1.770, p=0.079].



Graph 1. Age (years) of mothers by type of pregnancy

2. Number of births (parity) according to the type of pregnant women

Of the total number of pregnant women with physiological pregnancy (n=60), most of them had one birth (43.3%), followed by 3 or more births (33.3%), 2 births (20%) and no birth (3.3 %). Of the total number of pregnant women with preeclamptic pregnancy (n=60), most of them had one birth (41.7%), followed by 3 or more births (30.0%), 2 births (28.3%) and none without birth (0.0%).



Graph 2. Correlation between parity and type of pregnancy

Table 1. Age (years) of mothers according to type of pregnancy

The pregnant group	N	Minimum	Maximum	Average value	Standard Deviation	p- value
Physiological pregnancy	60	17.00	42.00	29.95	6.10	>0.05
Preeclamptic pregnancy	60	18.00	38.00	28.13	5.10	

Table 2. Correlation between parity and type of pregnancy

			The pregnant group		Total
			Physiological pregnancy	Preeclamptic pregnancy	
Parity	0	Number	2	0	2
		% of the pregnant group	3.3%	0.0%	1.7%
	1	Number	26	25	51
		% of the pregnant group	43.3%	41.7%	42.5%
	2	Number	12	17	29
		% of the pregnant group	20.0%	28.3%	24.2%
	3 and more	Number	20	18	38
		% of the pregnant group	33.3%	30.0%	31.7%
Total		Number	60	60	120
		% of the pregnant group	100.0%	100.0%	100.0%

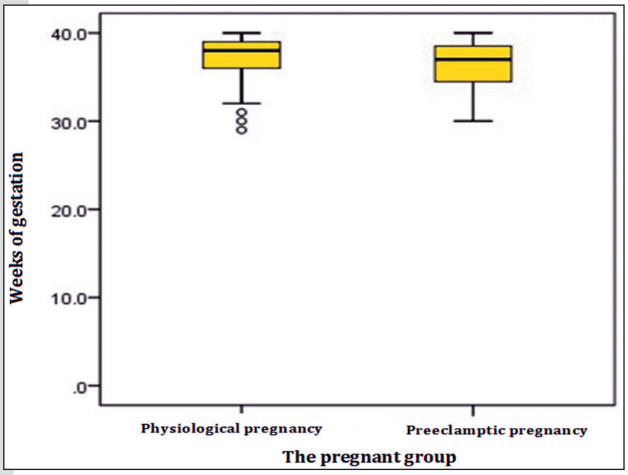
There is no statistically significant difference in parity frequency between physiological pregnancy and preeclamptic pregnancy. [χ^2 (3)=2,987, p=0,394].

3. Weeks of pregnancy of pregnant women according to the type of pregnancy

Pregnant women with preeclamptic pregnancy have lower median gestational values [Me=37.00 to 38.75)] compared to pregnant women with physiological pregnancy [Me = 38.00 (IQR = 36.00 to 39.00)].

There is a statistically significant difference.

There is no statistically significant difference in the median gestation between pregnant women with physiological and preeclamptic pregnancy [U=1455,500, z = -1.827, p = 0.068].



Graph 3. Gestational weeks according to the type of pregnancy

4. Body mass of the newborn according to the type of pregnant women

Pregnant women with preeclamptic pregnancy had newborns with a smaller body mass [Me=2220.00 g (IQR=2055.00 to 2350.00)] com-

Table 3. Weeks of gestation according to the type of pregnancy

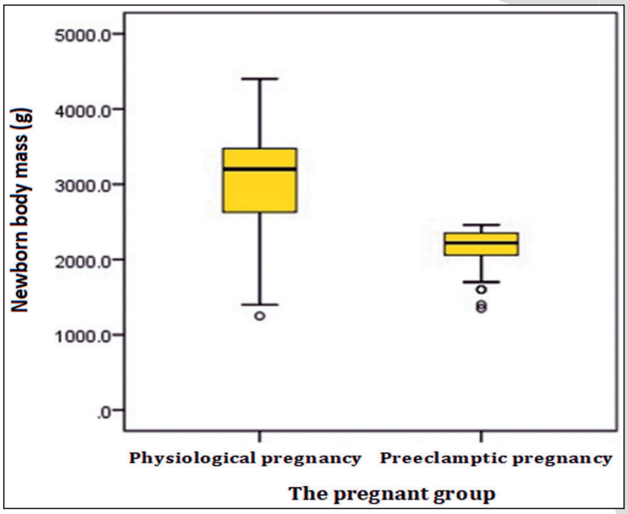
			The pregnant group	
			Physiological pregnancy	Preeclamptic pregnancy
Weeks of gestation	N		60	60
	Mean		37.03	36.32
	Std. Deviation		2.66	2.55
	Minimum		29.00	30.00
	Maximum		40.00	40.00
	Percentiles	25th	36.00	34.25
		50th (Median)	38.00	37.00
		75th	39.00	38.75
p- value			>0.05	

Table 4. Body mass (g) of the newborn according to the type of pregnancy

			The pregnant group	
			Physiological pregnancy	**Preeclamptic pregnancy**
Body mass of the newborn	N		60	60
	Mean		3010.00	2168.00
	Std. Deviation		670.81	261.49
	Minimum		1250.00	1350.00
	Maximum		4400.00	2460.00
	Percentiles	25th	2615.00	2055.00
		50th (Median)	3200.00	2220.00
		75th	3487.50	2350.00
p- value			<0.001	

pared to pregnant women with physiological pregnancy [Me=3200.00 g (IQR=2615.00 to 3487.50)].

There is a statistically significant difference in the median body mass among newborns in pregnant women with physiological and preeclamptic pregnancies [U=489,500, z=-6.883, p <0.001].



Graph 4. Body mass of the newborn (g) according to the type of pregnancy

5. Biparietal diameter (BPD) of fetuses in normal and preeclamptic pregnancy

IUGR fetuses have lower mean BPD values [Me=84.60 (IQR=80.00 to 86.40)] compared to fetuses with normal growth and development [Me=92.40 (IQR=82.33 to 93.50)].

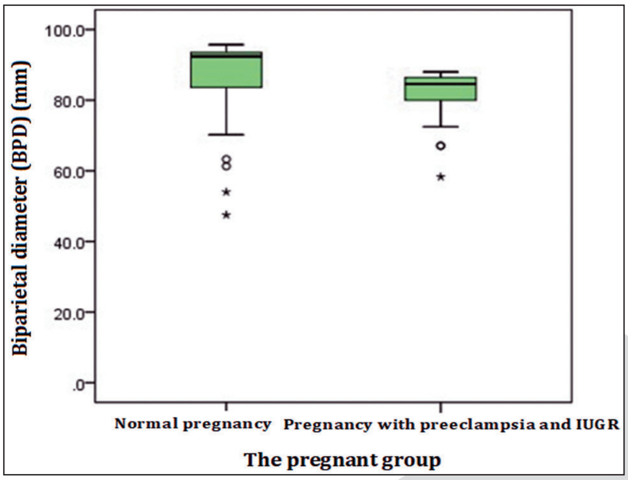
There is a statistically significant difference in the mean value of BPD between fetuses with growth retardation (IUGR) and fetuses with normal growth and development [U=884.000, z=-4.817, p<0.001].

6. Correlation of gestational week and biparietal diameter (BPD) of fetus with normal growth and IUGR

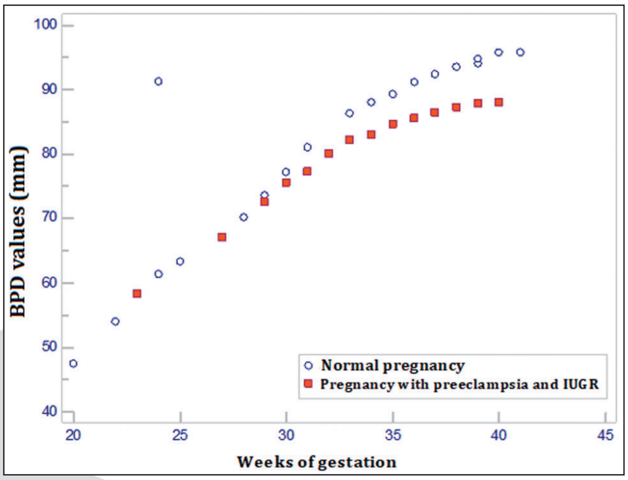
There is a statistically significant positive linear correlation between the values of gestational week and BPD in fetuses with normal development and growth (r_s=0.983; p<0.001).

Table 5. BPD values in normal and preeclamptic pregnancies with IUGR

			The pregnant group	
			Normal pregnancy	**Pregnancy with preeclampsia and IUGR**
Biparietal diameter (BPD) (mm)	N		60	60
	Mean		86.94	82.73
	Std. Deviation		11.24	6.03
	Minimum		47.50	58.30
	Maximum		95.70	88.00
	Percentiles	25th	82.33	80.00
		50th (Median)	92.40	84.60
		75th	93.50	86.40
p- value			<0.001	



Graph 5. BPD values in normal and preeclamptic pregnancies with IUGR.



Graph 6. Correlation between gestational weeks and fetal BPD.

There is a statistically significant positive linear correlation between gestational week and BPD values in fetuses with growth retardation (r_s=1.00; p<0.001).

7. Amniotic Fluid Index (AFI) in normal pregnancy and preeclamptic pregnancy

Growth retardation (IUGR) fetuses had lower median AFI values [Me=6.50 (IQR=4.50 to 11.00)]

Table 6. Correlation between gestational week and fetal BPD

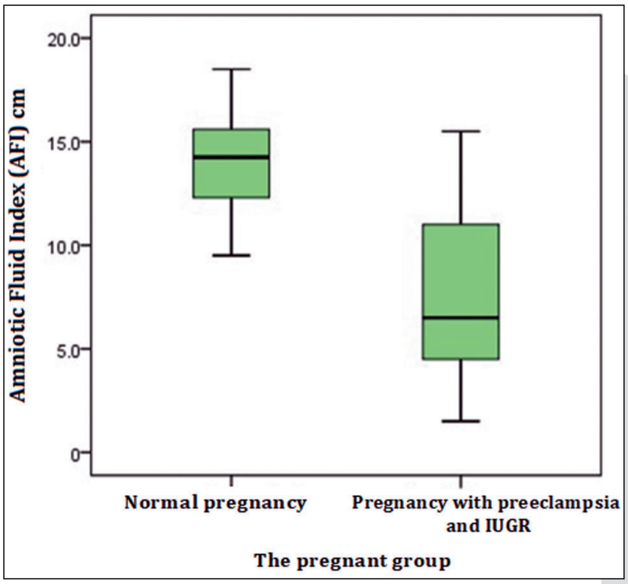
	The pregnant group			Weeks of gestation	BPD (mm)
Spearman's rho	Fetus with normal growth and develop-ment	Weeks of gestation	Correlation Coefficient	1.000	0.983**
			Sig. (2-tailed)	.	0.000
			N	60	60
		BPD (mm)	Correlation Coefficient	.983**	1.000
			Sig. (2-tailed)	0.000	.
			N	60	60
	Fetus with IUGR	Weeks of gestation	Correlation Coefficient	1.000	1.000**
			Sig. (2-tailed)	.	0.000
			N	60	60
		BPD (mm)	Correlation Coefficient	1.000**	1.000
			Sig. (2-tailed)	0.000	.
			N	60	60

Table 7. AFI values in normal and preeclamptic pregnancies with IUGR

Pregnancy with preeclampsia and IUGR			The pregnant group	
			Normal pregnancy	Pregnancy with preeclampsia and IUGR
Amniotic Fluid Index (AFI) cm	N		60	60
	Mean		14.3	7.56
	Std. Deviation		2.18	4.04
	Minimum		9.50	1.50
	Maximum		18.50	15.50
	Percentiles	25th	12.30	4.50
		50th (Median)	14.25	6.50
		75th	15.65	11.00
p- value			<0.001	

compared to fetuses with normal growth and development [Me=14.25 (IQR=12.30 to 15.65)].

There is a statistically significant difference in the AFI median values between fetuses with growth retardation (IUGR) and fetuses with normal growth and development [U=334.500, z=-7.696, p<0.001].

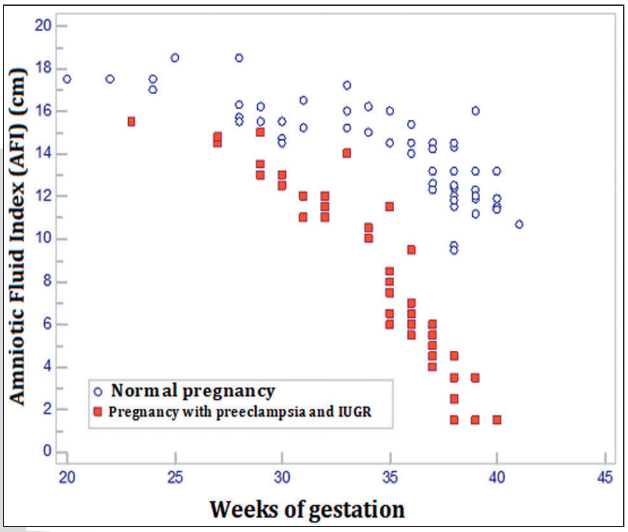


Graph 7. AFI values in normal and preeclamptic pregnancies with IUGR

8. Correlation of the week of pregnancy and the amniotic fluid index (AFI) of the fetus

There is a statistically significant negative linear correlation between the gestational week and AFI values in fetuses with normal development and growth (r_s=-0,853; p<0,001).

There is a statistically significant negative linear correlation between gestational week and AFI values in fetuses with growth retardation (r_s=-0,904; p<0,001).



Graph 8. Correlation of gestational week and AFI in the fetus

9. Lactate Dehydrogenase (LDH) in serum in physiological and preeclamptic pregnancy

Preeclamptic pregnant women have higher serum LDH values [Me = 509.50 to 796.25)] compared to normal pregnant women [Me = 356.50 U/L (IQR = 296.50 to 456.00)].

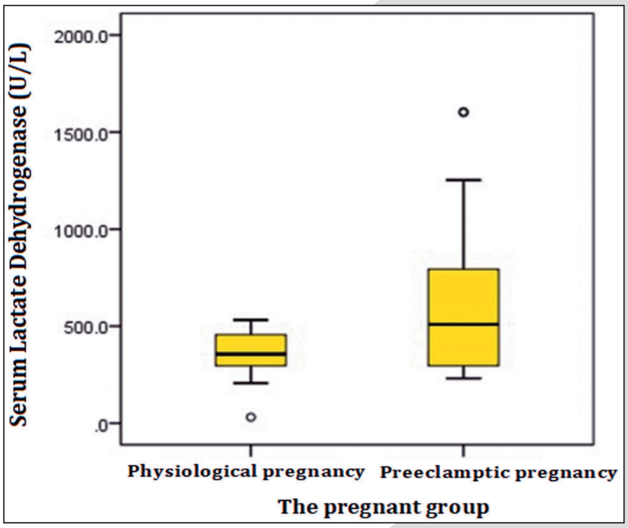
There is a statistically significant difference in the value of LDH in serum between pregnant women with physiological and preeclamptic pregnancy [U=1131,500, z = -3.509, p <0.001].

Table 8. Correlation of gestational week and AFI in the fetus

	The pregnant group			Weeks of gestation	AFI (cm)
Spearman's rho	Fetus with normal growth and development	Weeks of gestation	Correlation Coefficient	1.000	-0.853**
			Sig. (2-tailed)	.	0.000
			N	60	60
		Amniotic Fluid Index (AFI)	Correlation Coefficient	-0.853**	1.000
			Sig. (2-tailed)	0.000	.
			N	60	60
	Fetus with IUGR	Weeks of gestation	Correlation Coefficient	1.000	-0.904**
			Sig. (2-tailed)	.	0.000
			N	60	60
		Amniotic Fluid Index (AFI)	Correlation Coefficient	-0.904**	1.000
			Sig. (2-tailed)	0.000	.
			N	60	60

Table 9. Serum lactate dehydrogenase values (U/L) according to pregnancy type.

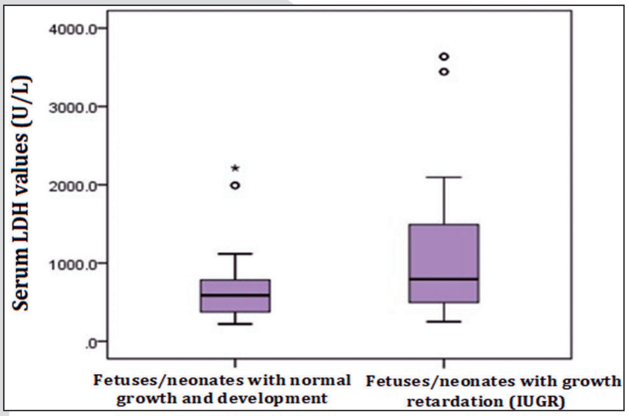
The pregnant group			The pregnant group	
			Physiological pregnancy	Preeclamptic pregnancy
Lactate dehydrogenase (U/L)	N		60	60
	Mean		364.72	590.92
	Std. Deviation		104.80	345.65
	Minimum		31.00	231.00
	Maximum		532.00	1605.00
	Percentiles	25th	296.50	297.00
		50th (Median)	356.50	509.50
		75th	456.00	796.25
p-value			<0.001	



Graph 9. Serum lactate dehydrogenase (U/L) values according to pregnancy type.

10. Lactate Dehydrogenase (LDH) in fetal/newborn serum

Fetuses/neonates with growth retardation (IUGR) have higher serum LDH values [Me=795.00 U/L (IQR=491.00 to 1490.00)] compared to fetuses/neonates with normal growth and development [Me=587.00U/L (IQR=376.00 to 783.00)]. There is a statistically significant difference in the mean serum LDH values between fetuses with growth retardation (IUGR) and fetuses with normal growth and development [U=1203.000, z=-3.135, p=0.002].



Graph 10. Serum LDH values in fetuses/neonates with growth retardation (IUGR) and fetuses with normal growth and development.

Table 10. Serum LDH values in fetuses/newborns with growth retardation (IUGR) and fetuses/newborns with normal growth and development

			Group of fetuses/neonates	
			Fetuses/neonates with normal growth and development	Fetuses/neonates with growth retardation (IUGR)
Lactate dehydrogenase (U/L)	N		60	60
	Mean		678.02	1179.95
	Std. Deviation		431.16	944.82
	Minimum		222.00	251.00
	Maximum		2213.00	3639.00
	Percentiles	25th	376.00	491.50
		50th (Median)	587.00	795.00
		75th	783.00	1490.00
p- value			<0.01	

Table 11. Correlation of LDH values in maternal and fetal/newborn serum

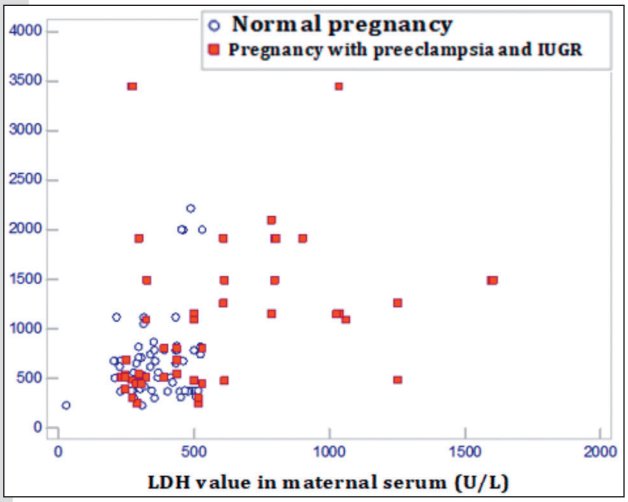
	The pregnant group			LDH (U/L) in mother	LDH (U/L) in the fetus/neonate
Spearman's rho	Normal pregnancy	Lactate dehydrogenase (U/L) in maternal serum	Correlation Coefficient	1.000	0.140
			Sig. (2-tailed)	.	0.285
			N	60	60
		Lactate dehydrogenase (U/L) in fetal serum	Correlation Coefficient	0.140	1.000
			Sig. (2-tailed)	0.285	.
			N	60	60
	Pregnancy with preeclampsia	Lactate dehydrogenase (U/L) in maternal serum	Correlation Coefficient	1.000	0.434**
			Sig. (2-tailed)	.	0.001
			N	60	60
		Lactate dehydrogenase (U/L) in fetal serum	Correlation Coefficient	0.434**	1.000
			Sig. (2-tailed)	0.001	.
			N	60	60

11. Correlation of biochemical parameters in maternal and fetal serum

11.1. Correlation of LDH values in maternal and fetal serum

There is no statistically significant linear correlation between maternal and fetal/newborn serum LDH values in normal pregnancy (r_s=0.140; p>0.05).

There is a statistically significant positive linear correlation between maternal and fetal/newborn serum LDH values in preeclamptic pregnancy (r_s=0.434; p<0.01).



Graph 11. Correlation of maternal and fetal/newborn serum LDH values

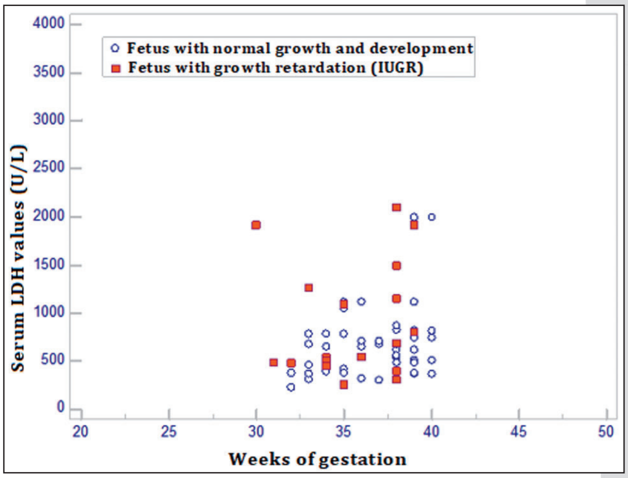
Table 12. Correlation of gestational week and LDH values in fetal/newborn serum

	The pregnant group			Weeks of gestation	Serum LDH (U/L)
Spearman's rho	Fetus with normal growth and development	Weeks of gestation	Correlation Coefficient	1.000	0.242
			Sig. (2-tailed)	.	0.062
			N	60	60
		Serum LDH (U/L)	Correlation Coefficient	0.242	1.000
			Sig. (2-tailed)	0.062	.
			N	60	60
	Fetus with IUGR	Weeks of gestation	Correlation Coefficient	1.000	0.247*
			Sig. (2-tailed)	.	0.034
			N	60	60
		Serum LDH (U/L)	Correlation Coefficient	0.274*	1.000
			Sig. (2-tailed)	0.034	.
			N	60	60

12. Correlation between weeks of pregnancy and LDH values in fetal serum

There is no statistically significant linear correlation between gestational week and serum LDH values in fetuses/newborns with normal growth and development (r_s=0.242; p>0.05).

There is a statistically significant positive linear correlation between gestational week and serum LDH values in fetuses/neonates with intrauterine growth retardation (r_s=0.274; p<0.05).



Graph 12. Correlation of gestational week and fetal/newborn serum LDH values.

5. Discussion

The mechanism of increased lactate dehydrogenase in pregnancy with preeclampsia is explained by the fact of endothelial cell damage and the release of these biochemical mediators (fibronectin, laminin). The release of cell degradation products stimulate the coagulation processes as well as the aggregation of platelets in the capillary walls, in this way the ratio of prostacyclin and thromboxane is disturbed. Due to these changes, vasoconstriction and tissue hypoxia appear in the liver of pregnant women with preeclampsia. In severe cases of preeclampsia, an increase in the activity of the hepatitis enzymes LDH, AST and ALT follows. There are data that lactate dehydrogenase and aminotransferases are increased in preeclampsia (26, 27, 28). The increase in the concentration of lactate dehydrogenase, aminotransferases and some other liver metabolites can be important markers that predict fetal growth retardation. The determination of lactate dehydrogenase, aminotransferase and cholesterol in pregnant women and newborns with growth retardation enable the differentiation and risk of perinatal complications due to hypoxia.

With the analysis of the Mann Whitney - U test, we found that there is a statistically significant difference in the AST values in serum between pregnant women with physiological and preeclamptic pregnancy [U=521.500, z=6.717, p<0.001]. Preeclamptic pregnancies had higher serum AST values [Me=27.00 U/L (IQR=22.00 to 34.00)] compared to physiological pregnancies [Me=14.00 U/L (IQR=11.00 to 19.00)].

The study (51) found that in preeclampsia there is an increased level of lactate dehydrogenase and aspartate aminotransferase.

Analysis using the Mann Whitney - U test has found that there is a statistically significant difference in serum LDH values between physiological pregnancies and preeclamptic pregnancies [U=1131.500, z=3.509, p<0.001]. Preeclamptic pregnancies had higher serum LDH values [Me=509.50 U/L (IQR=297.00 to 796.25)] compared to physiological pregnancies [Me=356.50 U/L (IQR=296.50 to 456.00)].

The increase in lactate dehydrogenase values is explained by the action of oxidative stress and the disorder of the synthesis of nitrogen monoxide, prostacyclin, coagulation factors, endothelin components, neuropeptides (10) which cause degenerative changes in hepatocytes with an increase in the level of lactate dehydrogenase.

Analysis by means of the Mann Whitney - U test has found that there is a statistically significant difference in serum LDH values between fetuses with stunted growth and fetuses with normal development and growth [U=1203.000, z=-3.135, p=0.002]. Growth retardation (IUGR) fetuses have higher serum LDH values [Me=795.00 U/L (IQR=491.00 to 1490.00)] compared to fetuses with normal growth and development [Me=587.00U/L (IQR =376.00 to 783.00)].

In fetal growth retardation due to hypoxia, which develops into preeclampsia, the activity of lactate dehydrogenase increases, which affects the glycolytic process of carbohydrate breakdown. In the conditions of hypoxia, damage to the liver cells follows, as well as the disorder of the permeability of the cell membrane, in some cases, the transfer of lactate dehydrogenase from the cell to the serum follows, as well as the increase in the level in the blood.

The other mechanism is explained by the fact that the increase in the level of LDH follows due to stress and hypoxia of the fetus as well as due to the increase in fetal cortisol from the adrenal gland. It is assumed that the high level of fetal prolactin stimulates the synthesis of lactate dehydrogenase in the fetal liver.

Our study with the help of the correlation coefficient according to Sperman has revealed that there is no significant difference in the linear correlation between the week of gestation and LDH values in the serum of the fetus/newborn with normal growth and development (r_s=0.242; p>0.05).

Our study by means of the correlation coefficient according to Spearman has found that there is a statistically significant difference of the positive linear correlation between the week of gestation and LDH values of the fetus/newborn with growth retardation IUGR (rs=0.274; p> 0.05).

The increase in the level of lactate dehydrogenase enzyme activity in the fetus with growth retardation is explained in this way: in the fetus with growth retardation (IUGR), the increase in the level of lactate dehydrogenase enzyme activity follows and is caused by hypoxia in the liver cells, transverse muscles and kidneys. Due to hypoxia, the permeability of the cell membrane is disturbed, as LDH molecules are transferred from the tissues to the plasma. It is known that the level of lactate dehydrogenase increases in the liver due to hypoxia, which occurs in fetuses with IUGR growth retardation. The cause of the appearance of preeclampsia is the disorder of the development of the placenta, which occurs due to the lack of trophoblast invasion in the walls of the spiral arteries of the placenta. Due to the lack of extravillous trophoblast invasion into the spiral arteries of the placenta, placental ischemia follows. Due to arterioplacental insufficiency, hypoxia and fetal growth retardation develop.

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Plasma Bioavailability of Vitamin D_3 Following Dietary Intake of OTM-2403-13 Insect Oil in Rats: A Controlled 14-Day Intervention Study

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Abstract

Background: The metabolic fate and systemic bioavailability of vitamin D_3 are profoundly shaped by the lipid matrix through which it is delivered, influencing micellar solubilization, enterocyte uptake, chylomicron secretion, and subsequent hepatic bioconversion. Insect-derived oils—rich in bioactive mono- and polyunsaturated fatty acids—have recently emerged as unconventional yet promising vectors capable of enhancing nutrient-lipid interactions and facilitating absorption.

Objective: This study sought to characterize, under controlled dietary conditions, the capacity of the vitamin-D_3–enriched insect oil OTM-2403-13 to restore and potentiate circulating 25(OH)D_3 concentrations in rats following nutritional depletion, compared with a vitamin D_3-deficient homologous lipid matrix.

Methods: Male Wistar rats were subjected to a 28-day depletion phase before receiving either: (1) a deficient control diet containing MO-2403-13 oil devoid of vitamin D_3, or (2) an intervention diet incorporating OTM-2403-13 delivering 22 µg/day/rat of vitamin D_3 for 14 days. Plasma 25(OH)D_3 was quantified using HPLC-DAD following standardized extraction and calibration.

Results: Animals receiving OTM-2403-13 exhibited a striking restoration of vitamin D status, with mean plasma 25(OH)D_3 rising from 6.9 ng/mL in deficient controls to 40.3 ng/mL ($p = 4.4\times10^{-11}$). Inter-individual variability was minimal within groups, indicating consistent absorption and metabolic handling.

Conclusion: OTM-2403-13 demonstrated substantial bioavailability, suggesting that insect-derived lipid matrices constitute a promising modality for dietary vitamin D_3 delivery and may provide translational applications for functional foods aimed at correcting insufficiency.

Keywords: Vitamin D3, bioavailability, insect oil, Tenebrio molitor, 25(OH)D_3, lipid matrix, nutrition, rodent model

1. Introduction

Vitamin D_3 remains a cornerstone of endocrine and immunometabolic physiology, with well-established roles in calcium homeostasis, bone remodeling, neuromuscular function, and innate immunity. Despite its biological centrality, vitamin D insufficiency is highly prevalent worldwide, driven by inadequate dietary intake, restricted sunlight exposure, and impaired metabolic activation. A substantial body of work has highlighted that the bioavailability of vitamin D_3 is strongly modulated by the surrounding lipid matrix, wherein fatty acid composition, emulsification behavior, micelle formation, and chylomicron assembly collectively influence its absorption efficiency.

Insect-derived oils have gained traction as sustainable lipid vectors exhibiting unique physicochemical properties, including favorable ratios of MUFA and PUFA, structured lipid configurations, and natural oxidative stability. These attributes may facilitate improved solubilization and transport of lipophilic micronutrients. OTM-2403-13, a Tenebrio-molitor–derived oil enriched in vitamin D_3, represents a novel candidate for enhancing dietary vitamin D delivery.

This study therefore examined the capacity of OTM-2403-13 to raise circulating 25(OH)D_3 levels under controlled nutritional conditions, following a standardized depletion protocol designed to mimic dietary insufficiency.

2. Materials and Methods

2.1 Animal Model

Eight-week-old male Wistar rats underwent a 28-day vitamin D_3 depletion period using a semi-purified diet containing 480 ng/100 g vitamin D_3 and 6.8% fat. Animals were randomized into two experimental groups (n = 8/group) under controlled environmental and lighting conditions.

2.2 Diets and Vitamin D_3 Exposure

The control group received pellets containing 7% MO-2403-13 oil, naturally devoid of vitamin D_3. The intervention group consumed pellets incorporating 7% OTM-2403-13, providing 1,652±149.5 µg vitamin D_3/100 g, equivalent to 22 µg/day/rat. All diets were offered ad libitum.

2.3 Plasma Collection and HPLC Analysis

Blood samples were collected at day 14 via terminal puncture. Plasma was analyzed for 25(OH)D_3 concentrations using high-performance liquid chromatography with diode-array detection (HPLC-DAD). Quantification employed external calibration curves constructed with certified standards. Statistical significance was assessed using Student's t-test with $\alpha = 0.05$.

3. Results

3.1 Plasma Vitamin D_3 Status Restoration

Following the intervention, plasma 25(OH)D_3 differed markedly between groups. Animals maintained on the vitamin-D_3–deficient MO-2403-13 oil remained severely depleted (mean 6.9 ng/mL).

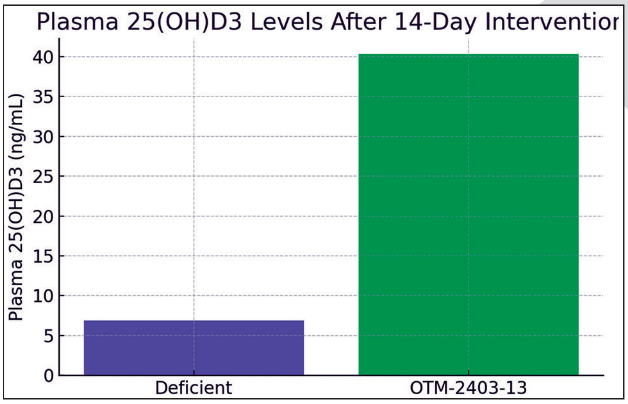

Figure 1. Plasma 25(OH)D_3 Levels After 14 Days

In contrast, rats receiving OTM-2403-13 exhibited a dramatic six-fold elevation (mean 40.3 ng/mL), with exceptional statistical support ($p = 4.4\times10^{-11}$).

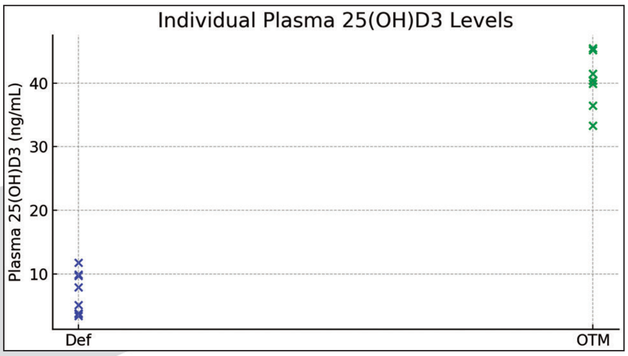

Figure 2. Individual Animal 25(OH)D_3 Values

Table 1. Individual Plasma 25(OH)D_3 Levels (ng/mL)

Animal	Deficient Control	OTM-2403-13
1	11.8	36.5
2	9.7	40.4
3	7.9	40.4
4	9.9	41.5
5	3.7	45.5
6	5.1	39.9
7	3.8	33.3
8	3.4	45.2

4. Discussion

The present investigation demonstrates, with high statistical robustness, that OTM-2403-13 is a highly bioavailable dietary source of vitamin D_3. The magnitude of the elevation in plasma 25(OH)D_3 is consistent with lipid-assisted enhancement of intestinal uptake, aligning with mechanistic literature that emphasizes the importance of micellar solubilization, enterocyte lipid trafficking, and hepatic hydroxylation kinetics.

Several factors may underpin the superior absorptive performance of OTM-2403-13:

(1) favorable MUFA-rich fatty acid composition enhancing micelle fluidity;
(2) potential synergistic interactions between insect lipids and intestinal lipid transporters;
(3) optimized partitioning into chylomicrons facilitating lymphatic entry; and
(4) the absence of inhibitory components commonly found in certain vegetable oils.

These findings resonate with recent nutritional science exploring alternative lipid matrices capable of improving the metabolic handling of fat-soluble vitamins.

Translationally, the results suggest that insect-derived oils may serve as viable fortification agents in food systems aiming to correct widespread vitamin D insufficiency. Further research - particularly long-term human studies - is warranted to elucidate dose-response dynamics, safety profiles, and matrix interactions in complex dietary environments.

5. Conclusion

Daily intake of 22 µg vitamin D_3 delivered through OTM-2403-13 insect oil produced a substantial restoration of circulating $25(OH)D_3$ following depletion. The bioavailability observed here underscores the translational potential of insect oils as functional lipid carriers for micronutrient fortification.

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Stress-relieving effects of Ashwa 30 (*Withania somnifera* extract): A randomised, double-blind, placebo-controlled study

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Abstract

Background and Aim: Exposure to stressful events activates the hypothalamic–pituitary–adrenal (HPA) axis, leading to increased cortisol release and subsequent physiological and emotional alterations. The present study aimed to evaluate the effect of Ashwa 30 on cortisol levels released in response to stress.

Methods: A one-week, randomized, double-blind, placebo-controlled study was conducted in forty healthy male participants (n=20 per group). Participants received either Ashwa 30 (30 mg) or placebo once daily for a week. On day 7, the Maastricht Acute Stress Test (MAST)—a

validated and reliable non-invasive procedure for activating the stress system—was administered. Saliva samples were collected 10 minutes post-MAST, and stress-related changes were assessed by comparing post-MAST salivary cortisol levels between the two groups.

Results and Conclusion: Ashwa 30 supplementation significantly reduced salivary cortisol levels following MAST exposure compared with placebo ($P = 0.03$), indicating an optimized stress response. The intervention was well tolerated, with no reports of major adverse effects.

Keywords: Ashwagandha, *Withania somnifera*, Stress, Cortisol, Acute Stress

Introduction

Stress, lack of energy or fatigue are one of major factors that exacerbate the risk of life-threatening health issues. The lack of a holistic approach in conventional medicine to deal with stress has led to a shift towards the use of herbal products/ nutraceuticals, lifestyle changes and other therapies like cognitive - behavioural therapy among people. (1). "Adaptogens" are ideal solutions to these problems owing to their comprehensive health benefits.

Ashwagandha (*Withania somnifera*), a popular herb and a potent adaptogen in the Ayurvedic system of medicine has a well-documented history of use for millennia. (2) (3). Ashwagandha is classified as a 'Rasayana' (rejuvenator) which helps to promote physical as well as mental health and improve longevity. (4). Various Ayurvedic texts such as Caraka-Samhita (1000 BC), Susruta-Samhita (1000 BC), Kasyapa-Samhita (600AD), highlight the multifaceted use of the root of Ashwagandha in numerous human ailments. (5). *W. somnifera* is used alone or in combination with other plants for treating various diseases. Ashwagandha, a perennial shrub (family Solanaceae), is cultivated in dry and scorching parts of tropical and subtropical zones of world. (6).

Stress can be defined as state in which the body's internal balance or homeostasis is disturbed or disrupted. (7). Any stimuli (physical or psychological stressor) which disrupts homeostasis result in a stress response. A stress response is generally a combination of the physiological and behavioural alterations that manifest while encountering a stressor. The biological response to stress is mediated through a complex network of multisystem mechanisms which lead to the activation of the Sympathetic-Adreno-Medullar System and Hypothalamic-Pituitary-Adrenal System, the 2 major pillars of the stress response (8). The sympathetic-adreno-medullar (SAM) axis, secretes noradrenaline and norepinephrine and the hypothalamus-pituitary-adrenal (HPA) axis, secretes glucocorticoids including cortisol as a stress response. (9).

Acute stress has significant implications across diverse occupational settings. In professions exposure to unpredictable and threatening situations demands rapid decision-making under intense physiological and psychological arousal. This acute stress response, mediated through activation of the autonomic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis, can impair cognitive flexibility, working memory, and fine motor skills, thereby affecting judgment, reaction time, and task execution. Beyond tactical occupations, acute stress is also prevalent in healthcare, education, and corporate sectors, where high workload, time pressure, and performance expectations can precipitate errors, reduce efficiency, and increase emotional exhaustion. Collectively, these effects underscore the importance of developing interventions to optimize stress regulation and preserve performance in both high-stakes and everyday work environments (10).

The current spike in the demand for ashwagandha has led to the emergence of root or root & leaf based ashwagandha products. This unmet demand calls for a standardised, root-only, low dose ashwagandha product with established efficacy and safety. The present study was designed to evaluate the effect of Ashwa 30, a unique low-dose Ashwagandha extract developed through Bioactive Optimization Technology, on cortisol release under acute stress conditions in healthy participants following seven days of supplementation.

Methods

Study design

The two-arm randomised, prospective, double-blind, placebo - controlled clinical study was conducted at Natural Remedies Private Limited, Bengaluru, India.

Recruitment and randomisation

After signing the informed consent, eligible participants were randomly allocated to Ashwa 30 or placebo group in 1:1 ratio through a simple randomisation method. To ensure sequence concealment, the randomization sequence was completed by a researcher not involved in participant recruitment and details of the bottle codes held with sponsor were revealed after data collection.

Participants

Inclusion criteria

Healthy male adults aged 25 -55 years were recruited after a routine health checkup. Participants who were willing to provide a personally signed and dated informed consent form with details of the study and willing to abstain from all dietary supplements were recruited.

Exclusion criteria

Participants suffering from or have a history of medical conditions such as but not limited to diabetes mellitus, hypertension, other cardiovascular diseases, gastrointestinal disorders, endocrine disorders, infectious diseases, acute or chronic pain conditions, neurological disorders, psychiatric disorders and skin conditions that prevent them from taking the test.

Participants were deemed ineligible if they were taking vitamin or mineral supplements, anxiolytics, antidepressants, or any treatment likely to affect salivary cortisol levels, including topical steroids. Individuals with a history of illicit drug use, major surgical interventions, current use of medications or food supplements for any disease or disorder or known hypersensitivity to the investigational product were excluded. In addition, participants who had experienced a significant life event within two weeks prior to enrollment or anticipated such an event during the study (e.g., bereavement, divorce, major changes in professional activities, or travel associated with jet lag) were not eligible. Shift workers, individuals working in extreme environmental conditions (e.g., cold rooms), those engaged in high levels of physical activity, or those with habitual consumption of more than three cups of coffee in the morning or more than three alcoholic drinks per day were excluded. Further restrictions required that participants maintain consistent dietary, sleep, and exercise routines throughout the study period.

Intervention

Withania somnifera also known as Ashwagandha (Family: Solanaceae) roots were sourced from southern parts of India. The authentication was done, and voucher specimen number 128101 is kept in the Herbarium of the Foundation for Revitalisation of Local Health Traditions (FRLHT) in Bengaluru.

The test substance used was a proprietary extract (Pending patent: 202541047628) derived from the roots of *Withania somnifera*. It was extracted with ethanol and water to achieve a standardization of withanolides (>15% w/w determined using a modified USP HPLC method). In addition to it, the extract was also standardized to ATP active fraction (>15% w/w).

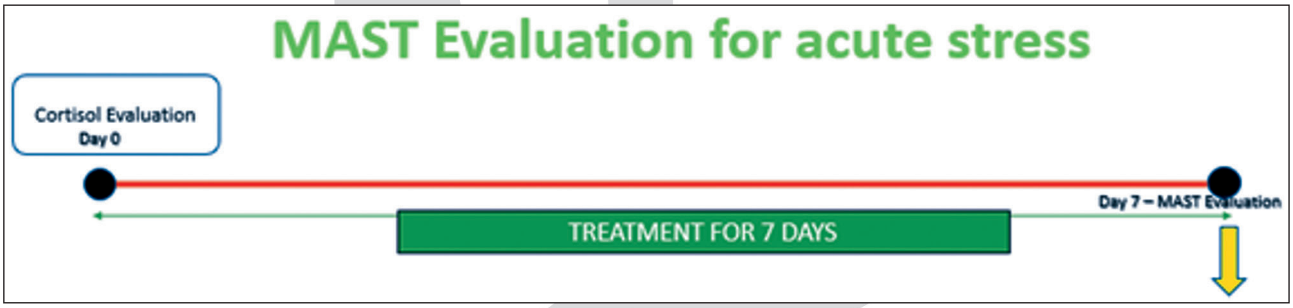
Ashwa 30 capsules containing 30 mg of standardised root- only Ashwagandha extract (Ashwa-30™) were used as the test product. Capsules containing microcrystalline cellulose and syloid 244 FP were used as the placebo. Both Ashwa 30 and placebo capsules were manufactured in a Good Manufacturing Practice (GMP)-certified facility. The test products complied with the United States Pharmacopeia (USP) requirements for dietary supplements, including limits for microbial contaminants, heavy metals, residual pesticides, aflatoxins and residual solvents. Each bottle dispensed to subjects contained capsules of either Ashwa 30 or placebo and was labelled with a unique randomization number. Subjects were instructed to take one capsule daily in the morning, either before or after food, for 7 days *(Figure 1)*. The Ashwa 30 and placebo capsules prepared in such a way to make sure that they were identical in appearance and were packaged in a manner to ensure blinding.

Outcome measure

Maastricht acute stress test - MAST

MAST is a simple, swift and non-invasive valid and reliable procedure designed to stimulate a stress response (11) (12).

MAST begins with a 5min preparation/ room acclimatization phase. The participants are briefed about the upcoming task and the instructions are given. During the 12min MAST *(Figure2),* participants were required to place their hand in cold water at 7◦C and complete an arithmetic task (counting backward by 7s) for alternating time intervals. Five hand immersion trials were alternated with four mental arithmetic tasks. 5 ml of saliva was collected at Day 0, Day 7 before MAST and Day 7 after MAST for cortisol evaluation (from 2.30 pm to 5 pm due to the diurnal cycle of cortisol). On the day of analysis, samples were thawed and precipitated mucins and particulate debris were separated by centrifugation at 1,500 g for 25min at room temperature. An aliquot of the supernatant was used for the measurement of cortisol. Salivary cortisol was measured using Electrochemiluminescence Immunoassay (ECLIA).



CPT- Cold Pressor Test, MAT- Mental Arithmetic Test

Figure 1. Trial protocol

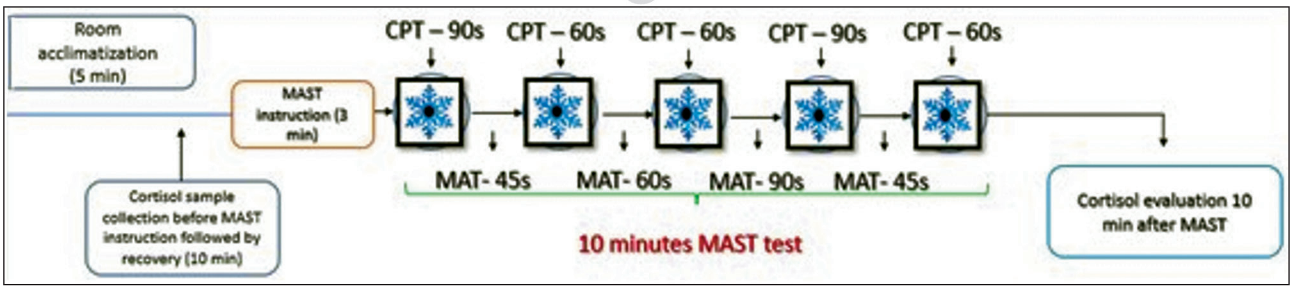


Figure 2. MAST test Procedure

Salivary cortisol levels before and after acute stress induction was measured as outcome of the study. Salivary measure was chosen as it is non-invasive, inducing minimal or no stress and also is a validated method to measure HP axis in stress response (13)

Safety assessments

Safety assessments included adverse events monitoring, vital signs and general physical examination. AEs were monitored continuously throughout the study. Vital signs (SpO2, blood pressure, respiratory rate, pulse rate, and body temperature) and physical examinations were performed at each study visit.

Statistical analysis

Quantitative variables were reported as mean ± standard deviation. Within-group comparisons were performed using a paired t-test or Wilcoxon signed-rank test, depending on data distribution. Between-group comparisons were conducted using an unpaired t-test or Mann-Whitney U test. Normality tests (Kolmogorov-Smirnov or Shapiro-Wilk) were conducted to assess data distribution assumptions. All statistical tests were two-sided, with a significance threshold set at $p<0.05$.

Results

Out of 44 adult men who were willing to participate in the study, 40 participants were eligible based on the inclusion and exclusion criteria. Participants were assigned either to Ashwa 30 or placebo group.

Salivary cortisol levels

Table. 1 depicts the changes in the cortisol levels at Day 7 before and after MAST in both Ashwa 30 and placebo groups. There was no significant difference with respect to salivary cortisol levels of Ashwa 30 and placebo pre-MAST. The rise is salivary cortisol post MAST was significantly less compared to placebo. Although there is a significant increase in the salivary cortisol levels in the Ashwa 30 group after MAST compared to the before MAST levels, the increase is highly significant in the Placebo group.

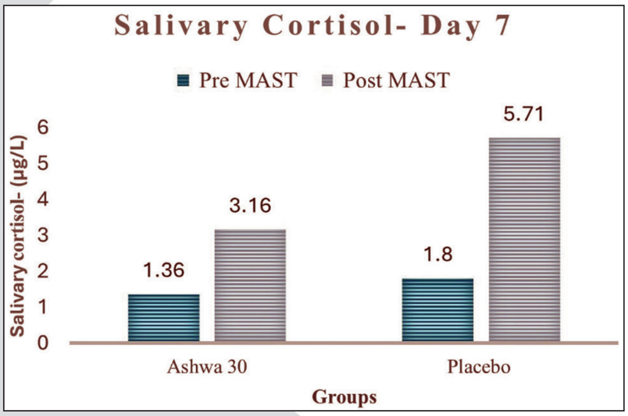


Figure 3. Effect of Ashwa 30 on salivary cortisol

Safety assessments

No adverse events were reported during the study. No abnormalities were observed in the vital signs and physical examinations.

Discussion

Acute stress is known to significantly impair performance and decision-making, reduce efficiency, and induce exhaustion; moreover, repeated exposure over the long term can have profound adverse consequences on overall health and functioning.

This study aimed to analyse the efficacy and safety of Ashwa 30 in managing acute stress. The study results revealed that Ashwa 30 extract signi-

Table 1. Salivary cortisol levels- pre and post MAST

Salivary cortisol (µg/L) - Day 7			
	Pre MAST	**Post MAST**	**p value** *(within group)*
Ashwa 30	1.36 ± 0.96	3.16 ± 2.02	0.02*
Placebo	1.80 ± 0.73	5.71 ± 3.83	0.0008*
p value *(Ashwa 30 vs Placebo)*	0.1856	0.0387#	

** P < 0.05 = statistically significant in within group, otherwise the difference was not statistically significant.*

 # P < 0.05 = statistically significant in between groups, otherwise the difference was not statistically significant.

Data were analyzed using an unpaired t-test for between-group comparisons and a paired t-test for within-group comparisons.

ficantly reduced the salivary cortisol levels post-acute stress induction. While there are multiple studies on Ashwagandha from root /root and leaf extracts studied the effects of intervention on cortisol in chronic stress (14). This is the first study that investigated the effect of Ashwa 30 on cortisol levels in acute settings.

It is noteworthy to highlight that the cortisol awakening response was not altered by Ashwa 30 after acute stress induction, but the increase was significantly lesser compared to placebo. This is beneficial because stress response is essential for survival. Thus, Ashwa 30 aids in optimizing stress regulation and preserves performance in work environment & other high-stake environments.

Ashwa 30 possibly reduces stress by modulating the hypothalamic-pituitary-adrenal (HPA) axis and thereby reducing the cortisol levels. The well-established relationship between stress and neuropsychiatric disorders, highlights the speciality of ashwagandha's anti-stress activity and its potential health benefits. (15).

A key strength of this study is its focus on examining the activity of Ashwa 30 in stress management after a short, 7-day consumption period.

While Ashwa 30 demonstrated significant efficacy in managing acute stress after 7 days, further studies with a larger subject pool and longer durations are needed to fully substantiate its efficacy and safety.

Conclusion

In conclusion, standardised root-only, Ashwa 30 ™ proves its efficacy in managing acute stress by significantly reducing salivary cortisol levels. No serious adverse events were observed during the study.

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Plasma Bioavailability of Vitamin D_3 Delivered Through Mealworm Oil (D3MO-2403-13): An In Vivo Assessment Using a Rat Depletion-Repletion Model

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Abstract

Background: Vitamin D_3 bioavailability varies widely according to lipid matrix characteristics. This study evaluates the plasma availability of vitamin D_3 delivered through a highly concentrated mealworm oil (D3MO-2403-13), compared with a vitamin-D–deficient reference oil.

Methods: Male Wistar rats underwent a 28-day depletion phase followed by 14 days of dietary intervention. One group received a vitamin D–deficient diet (MO-2403-13), while the intervention group received a daily oral gavage containing 22 µg vitamin D_3 from D3MO-2403-13. Plasma 25(OH)D_3 was quantified by HPLC-DAD.

Results: Mean plasma 25(OH)D_3 increased from 6.9 ng/mL in controls to 52.3 ng/mL in the D3MO-2403-13 group ($p = 2.2\times10^{-10}$). Individual values consistently demonstrated strong vitamin D_3 uptake and hepatic conversion.

Conclusion: Mealworm-derived vitamin D_3 oil is highly bioavailable, yielding a 7.5-fold increase in circulating 25(OH)D_3. These findings support the use of entomological oils as efficient vehicles for nutritional fortification.

Key words: Vitamin D_3; bioavailability; mealworm oil; entomological lipids; nutritional fortification; rat model.

1. Introduction

Vitamin D_3 plays indispensable roles in skeletal physiology, muscle function, metabolic regulation, and immune homeostasis. Its main circulating metabolite, 25-hydroxyvitamin D_3 (25(OH)D_3), is widely recognized as the most reliable marker of vitamin D status. Because dietary vitamin D_3 depends on lipid-based digestive processes, its absorption is shaped by the surrounding fatty acid matrix, micellar solubilization dynamics, and post-absorptive lipid transport mechanisms. These factors collectively influence how efficiently vitamin D_3 enters enterocytes and is subsequently delivered through the lymphatic system for hepatic conversion.

In recent years, edible insect oils have received attention as promising alternative lipid matrices due to their favorable fatty acid compositions, oxidative stability, and digestibility. Mealworm (Tenebrio molitor) oil, in particular, is rich in monounsaturated fatty acids and exhibits physicochemical properties that may facilitate greater bioefficacy of fat-soluble micronutrients.

The present study evaluates the plasma bioavailability of vitamin D_3 delivered through a highly concentrated mealworm oil formulation (D3MO-2403-13) using a controlled depletion–repletion rat model. This design enables precise quantification of repletion efficiency following a standardized period of dietary vitamin D deficiency.

2. Materials and Methods

This study employed a depletion–repletion design to characterize vitamin D_3 bioavailability from mealworm oil. Male Wistar rats were maintained on a vitamin D–deficient diet for 28 days to normalize circulating 25(OH)D_3 levels across animals. Following depletion, rats were assigned to either a control group receiving MO-2403-13 (vitamin-D–deficient oil) or to an intervention group receiving a daily oral gavage of D3MO-2403-13 delivering 22 µg vitamin D_3 for 14 consecutive days.

All animals were monitored for body weight, general health, and dietary intake during the intervention period. Blood samples were collected at the end of the 14 days. Plasma 25(OH)D_3 was quantified using HPLC-DAD following saponification and organic extraction. Calibration was performed using certified standards. Statistical analyses were conducted using two-tailed Student's t-tests with significance set at $p < 0.05$.

3. Results

Supplementation with D3MO-2403-13 produced a marked elevation in circulating 25(OH) D_3. After 14 days, the control group remained deficient with a mean concentration of 6.9 ng/mL, whereas the supplemented group reached 52.3 ng/mL ($p = 2.2 \times 10^{-10}$). This 7.5-fold increase demonstrates efficient gastrointestinal absorption and hepatic conversion.

Individual results showed uniformly strong responses, indicating robust biological uptake across animals and minimal inter-individual variability. These findings confirm that vitamin D_3 delivered in mealworm oil is highly bioavailable.

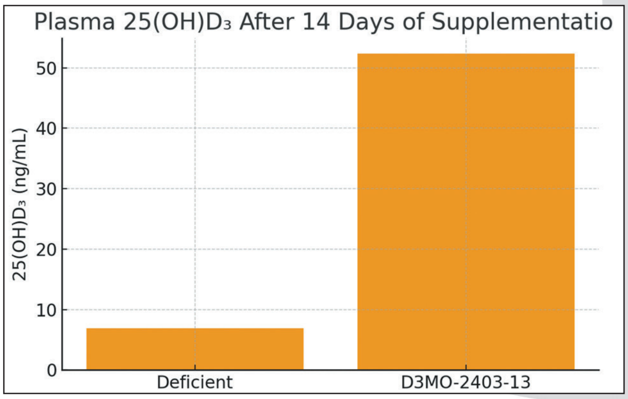

Figure 1. Mean 25(OH)D_3 Levels

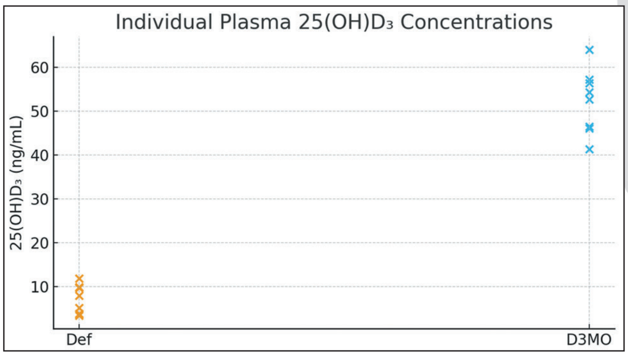

Figure 2. Individual 25(OH)D_3 Values

Table 1. Individual plasma concentrations of 25(OH)D_3

Animal	Deficient Control	D3MO-2403-13
1	11.8	46.5
2	9.7	52.7
3	7.9	41.3
4	9.9	64.0
5	3.7	57.2
6	5.1	56.4
7	3.8	46.1
8	3.4	54.3

4. Discussion

The results demonstrate that vitamin D_3 delivered through D3MO-2403-13 mealworm oil is absorbed efficiently and supports rapid repletion of circulating 25(OH)D_3 following dietary depletion. The magnitude of increase observed in this model is consistent with the hypothesis that mealworm oil's monounsaturated fatty acid profile may promote more favorable micellar incorporation and lipid-mediated transport of cholecalciferol.

These findings complement existing literature showing that lipid structure, unsaturation level, and matrix polarity contribute meaningfully to the absorption of fat-soluble nutrients. Oils with balanced MUFA content often exhibit enhanced bioaccessibility due to improved emulsification efficiency and compatibility with intestinal lipid-handling pathways.

As interest grows in sustainable nutritional lipids, the present data support the feasibility of using insect-derived oils as effective delivery systems for micronutrient fortification. Future investigations should consider dose–response comparisons, long-term metabolic effects, and translational evaluation in human studies.

5. Conclusion

Administration of 22 µg/day vitamin D_3 through D3MO-2403-13 mealworm oil substantially increased circulating 25(OH)D_3 in a depletion–repletion model, demonstrating excellent bioavailability and metabolic activation. Mealworm oil appears to be a promising lipid carrier for nutritional applications seeking enhanced delivery of vitamin D_3 and other fat-soluble micronutrients.

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Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 key words.

Key words: Camera ready paper, Journal.

Introduction

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Instructions for the authors

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Table 1. Page layout description

Paper size	A4
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Right margin	18 mm
Column Spacing	5 mm

Regular paper may be divided in a number of sections. Section titles (including references and acknowledgement) should be typed using 12 pt fonts with **bold** option. For numbering use Times New Roman number. Sections can be split in subsection, which should be typed 12 pt *Italic* option. Figures should be one column wide. If it is impossible to place figure in one column, two column wide figures is allowed. Each figure must have a caption under the figure. Figures must be a resolution of 300 DPI, saved in TIFF format, width 10 cm min. For the figure captions 12 pt *Italic* font should be used. (1)

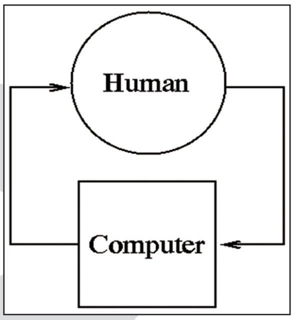

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

These and the Reference headings are in bold but have no numbers.

References

1. *Sakane T, Takeno M, Suzuki N, Inaba G. Behcet's disease. N Engl J Med 1999; 341: 1284–1291.*
2. *Stewart SM, Lam TH, Beston CL, et al. A Prospective Analysis of Stress and Academic Performance in the first two years of Medical School. Med Educ 1999; 33(4): 243- 50.*

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