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Research Article

Effectiveness of Earthworm (*Lumbricus rubellus* Hoffmeister) and Cinnamon (*Cinnamomum burmannii* (Nees & T. Nees) Blume) Combination Capsule as Herbal Preparation in Reducing Cholesterol and Blood Sugar Levels

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ABSTRACT

Cardiovascular diseases, including coronary heart disease and heart attack symptoms, are the leading causes of death worldwide. High cholesterol levels and blood sugar levels contribute significantly to these diseases. Therefore, this study aimed to determine the effectiveness of combining capsules of earthworm (*Lumbricus rubellus*) and cinnamon (*Cinnamomum burmannii*) in lowering cholesterol and blood sugar levels. After conducting pre-clinical trials, clinical trials were further carried out in humans. Cholesterol and blood sugar levels were measured for 14 days in Phases 1 and 2. Statistical data analysis was conducted using the Shapiro-Wilk normality test, paired t-hypothesis test, Wilcoxon test, and frequency analysis. The results showed that in Phase 1, there were significant differences in volunteers' cholesterol and blood sugar levels with no clinical symptoms. The most significant decrease in cholesterol levels in female volunteers occurred at the age of 21-30 years, at 25.56%, while the most significant reduction in blood sugar levels occurred at 41-50 years, at 41.50%. On the other hand, the most significant decrease in cholesterol levels in male volunteers occurred at the age of 41-50 years at 33.08%, and the most significant reduction in blood sugar levels occurred at the age of >60 years at 38.87%. The most significant content in the combination capsule was fatty acids and cinnamaldehyde. In conclusion, consuming a combination of earthworm and cinnamon capsules for 14 days caused a reduction in cholesterol and blood sugar levels.

1. Introduction

Cardiovascular diseases, including coronary heart disease and heart attack symptoms, are the leading causes of death worldwide. High cholesterol levels and blood sugar levels contribute significantly to these diseases. Normal cholesterol and sugar levels in the blood are estimated at 200 mg/dl. Exceeding the normal levels might lead to hypercholesterolemia and diabetes mellitus, respectively. According to the World Health Organization (WHO), in 2012, about 50% of heart attacks in individuals were caused by high levels of cholesterol in the blood. International Diabetes Federation (IDF) Atlas in 2021 also stated that Indonesia ranked fifth globally in diabetes prevalence with 19.47 million cases. This number was also predicted to reach 28.57 million in 2045.

Some natural ingredients can potentially reduce blood cholesterol and blood sugar levels to minimize the prevalence of hypercholesterolemia and diabetes mellitus. According to various studies, natural ingredients such as earthworms and cinnamon can lower blood cholesterol and blood sugar levels. Auliah (2008) reported that earthworms contain very high protein and amino acid levels capable of binding excess cholesterol in the blood (Marzuki *et al.* 2020). Emilda (2018) states that essential amino acids in earthworms improve the function of β

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cells in the pancreas to produce antihyperglycemic insulin. Soemardini *et al.* (2011) also reported reduced blood glucose levels, total cholesterol, triglyceride levels, and increased high-density lipoprotein (HDL) levels. In the study by Amri (2023), the average blood sugar level after being treated with cinnamon flour was 202.38 mg/dl, with a standard deviation of 36,739 mg/dl. This value was considered higher than the control group, with average blood sugar levels of 323,75 mg/dl and a standard deviation of 94,406 mg/dl. In a pre-clinical study conducted by Noor (2021), combining earthworm and cinnamon flour with a ratio of 1:1 reduced glucose levels in hyperglycemic rats (*Rattus norwegians*) and combining these two herbal materials obtained a synergic effect on cholesterol and blood sugar levels.

Traditional medicines derived from natural ingredients are often subjected to a series of tests, namely pre-clinical trials on test animal samples and clinical trials on humans. The clinical trials must follow the Helsinki Declaration, which consists of four phases. In the first phase, healthy volunteers are tested to obtain the same results as experimental animals. In the second phase, the drug material is tested on a specific patient and observed for efficacy in treating the disease. The third phase entails testing the effectiveness and safety of new drugs compared to existing ones. Once a candidate drug is proven to be efficacious and has benefits similar to or better than the comparison drug, it can be produced legally, marketed under a trade name, and prescribed by a doctor. In the fourth phase, after the drug is sold, a post-marketing study observes the patient's condition, age, and race. However, when the therapeutic effect is proven to be harmful in the long term, the drug can be withdrawn from circulation (Sudrajat 2006).

Based on the discussion above, this study aimed to determine the effectiveness of combining capsules of earthworm and cinnamon in lowering cholesterol and blood sugar levels. The experimental method was administering a combination capsule of earthworm and cinnamon to several volunteers with a limited clinical trial method. Cholesterol and blood sugar levels were measured for 14 days in Phases 1 and 2. Statistical data analysis starts from the Shapiro-Wilk normality test, paired t-hypothesis test, and Wilcoxon test.

2. Materials and Methods

The experimental procedures were approved by the Faculty of Public Health Universitas Hasanuddin approved (Approval ID: 10979/UN.4.14.1/TP.01.02/2022). Parametric data were obtained from an *in vivo* study to determine the effectiveness of combining capsules of earthworm and cinnamon in reducing blood cholesterol and blood sugar levels. The raw materials were obtained from Earthworm Farming Centre CV Raj Organik and PT Bioindonegeri, Malang, East Java, Indonesia. All raw materials were air-dried for 5-6 hours, and the water contained was about 50%. Subsequently, drying was carried out using a microwave in medium heat power (500-800 watts) for 10 minutes, and the water contained was reduced to 8-10%. The raw materials with a fragile texture were then milled into powder using a blender.

The powder was filtered through an 80-mesh sieve and filled into capsule shells made of gelatin with 500 mg capacity (250 mg earthworm: 250 mg cinnamon, ratio 1:1). The material was placed in a plastic package with silica gel and then stored in the storage room with a temperature of 25°C and humidity of 45%. Methanol and *n*-hexane were used in sample preparation for phytochemical analysis using GC-MS. Specifically, methanol was used for extraction, while *n*-hexane was used as a solvent in the fractionation process. Blood lancets, alcohol swabs, and blood strips Easy Touch GCU (glucose, cholesterol, uric acid) were used for blood sampling to measure cholesterol levels.

2.1. Chemical Content Analysis of Earthworm and Cinnamon Extract

Extraction was then done using the sonication method until a concentrated extract was produced. Subsequently, fractionation was carried out, producing a hexane fraction, which was analyzed using the Gas Chromatography-Mass Spectrophotometry Analysis (GC-MS) method. This analysis aimed to determine the bioactive compounds in earthworm and cinnamon extracts (Mahmiah *et al.* 2017).

A 1 µL hexane fraction was injected into GC-MS to analyze different compounds. Instrument and chromatography conditions were performed on the GC-MS QP2010 Ultra Shimadzu system. The column used was SH-RXi-5Sil MS with a length of 30 m, a diameter of 0.25 mm, and oven temperature ranging from 100 to 200°C. The temperature increase rate was 15°C/min, the flow rate was 1.0 ml/min, and the split ratio was 10:1. The eluted component was detected on the mass detector. Furthermore, the spectrum of known compound components was stored in the NIST library, determined by name and molecular weight, and included in the class of compounds such as terpenoids, alkaloids, flavonoids, phenols, and fatty acids (Mahmiah *et al.* 2017).

2.2. Limited Clinical Trials

The limited clinical trial in this study consisted of two phases, namely Phases 1 and 2, with different volunteers. Phase 1 clinical trial subjects were healthy volunteers with no symptoms of high cholesterol and blood sugar levels. Meanwhile, the subjects for Phase 2 clinical trials were volunteers who had high cholesterol and blood sugar levels after a direct measurement. Volunteers who were pregnant or had kidney failure were excluded.

Phase 1 was conducted to examine the safety of herbs on healthy volunteers. Observations included a decrease in cholesterol and sugar levels after administering a combination capsule to 20 volunteers with a specific age range of adolescents, adults, and the elderly. The clinical effects under study were nausea, bloating, fatigue, heartburn, dizziness, anorexia, and vomiting.

Phase 2 examined the effectiveness of combining capsules in volunteers with high cholesterol and blood sugar levels in the same age range as in Phase 1. Clinical trials in Phase 2 were carried out by administering combination capsules without control (uncontrolled trial) to observe the decrease in each volunteer's cholesterol and blood sugar levels. The dosage used was two capsules twice daily for 14 days and observation every 7 days in 20 volunteers (n = 20) following a pre-clinical study by Noor (2021), who determined the effect of combining earthworm and cinnamon extract in reducing blood sugar levels of rats *Rattus norwegians*. The observation showed that the absolute dose for combining earthworm (20 mg) with cinnamon extract (20 mg) was the best value for reducing blood glucose levels on day 7 and day 14. As shown in Table 1, rats (200 g bw) had a conversion factor 56.0 compared to the human dose.

The human dose formula is as follows:

$$\text{Animal absolute dose} \times \text{conversion factor}$$
$$(20 \times 56.0)\ \text{mg} = 1{,}120\ \text{mg}$$

Based on the results, the pharmacological effect of a drug that appears in humans at a dose of 1,120 mg/70 kg is the same as in rats at a dose of 2 mg/200 g from a similar drug. The appropriate dose of earthworm and cinnamon extract in the human body is >2000 mg/day, amounting to two capsules (500 mg) twice daily.

Cholesterol and sugar levels were measured on days 0, 7, and 14. Inclusion criteria include patients with symptoms of disease, using potent drugs continuously (patients of high blood pressure, diabetes mellitus, mental diseases such as schizophrenia), alcoholics, and have high SGPT/SGOT level analysis results. Meanwhile, the exclusion criteria were pregnant patients with kidney problems. Another general criterion is the age between 20 and 70 years and willingness to participate in this study.

2.3. Statistical Analysis

Parametric data generated from the analysis of blood cholesterol levels were first tested for normality using the Shapiro-Wilk test. When the data distribution was expected, a paired t-test was conducted at a confidence level of 95% (α = 0.05). However, the Wilcoxon Test was performed when data distribution was not expected. Frequency analysis was further conducted to determine the reduction in cholesterol levels using SPSS (Statistical Program for Social Science) for Windows.

Sample size determination/justification was performed using power analysis with G*Power Software for Windows. The statistical test was the difference between two independent means (two groups). The type of analysis was A Priori, calculating the required sample size based on predefined values of α, power, and effect size (r) (Kang 2021).

3. Results

3.1. Chemical Content of Earthworm and Cinnamon Extract

GC-MS analysis showed that fatty acids were the most significant compounds in the hexane fraction of earthworm and cinnamon. Table 2 shows that the

Table 1. Surface area ratios of some common laboratory species and man

Marker	Mouse (20 g)	Rat (200 g)	Guinea pig (400 g)	Rabbit (1.5 kg)	Cat (2 kg)	Monkey (4 kg)	Dog (12 kg)	Man (70 kg)
Mouse (20 g)	1.0	7.0	12.25	27.8	29.7	64.1	124.2	387.9
Rat (200 g)	0.14	1.0	1.74	3.9	4.2	9.2	17.8	56.0
Guinea pig (400 g)	0.08	0.57	1.0	2.25	2.4	5.2	10.2	31.5
Rabbit (1.5 kg)	0.04	0.25	0.44	1.0	1.08	2.4	4.5	14.2
Cat (2 kg)	0.03	0.23	0.41	0.92	1.0	2.2	4.1	13.0
Monkey (4 kg)	0.016	0.11	0.19	0.42	0.45	1.0	1.9	6.1
Dog (12 kg)	0.008	0.06	0.10	0.22	0.24	0.52	1.0	3.1
Man (70 kg)	0.0026	0.018	0.031	0.07	0.076	0.16	0.32	1.0

Table 2. The majority of compounds found in earthworm and cinnamon extracts

Material	Peak#	Area%	Compounds	R Time
Earthworm Extract	6	17.47	dodecanoic acid	12. 558
	16	22.15	z-11-pentadecenol	14.708
	17	10.10	tetradecanoic acid	14.938
	37	5.09	9,12-octadecadienoic acid (z,z)-	22.342
	38	4.03	oleic acid	22.460
	39	4.59	9-octadecenoic acid (z)-	22.533
	40	4.29	octadecanoic acid	22.966
	68	11.69	17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17 -tetradecahydro-1h-cyclopenta[a]phenant	40.239
Cinnamon Extract	5	25.53	2-propenal, 3-phenyl-	8.728
	13	11.11	dodecanoic acid	12.506
	20	8.40	z,z-8,10-hexadecadien-1-ol	14.637
	21	9.27	tetradecanoic acid	14.892
	36	4.46	9,12-octadecadienoic acid	22.551

total fatty acids contained in the hexane fraction of earthworms amounted to 45.57%. These include lauric acid (dodecanoic acid) 17.47%, tetradecanoic acid 10.10%, linoleic acid (9,12-octadecadienoic acid (Z,Z)-) 5.09%, oleic acid derivative (9-octadecenoic acid (Z)) 4.59%, octadecanoic acid by 4.29%, and oleic acid (oleic acid) 4.03%. Meanwhile, fatty acids contained in cinnamon extract amounted to 24.84%, including 11.11% lauric acid (dodecanoic acid), 9.27% tetradecanoic acid, 4.46% linoleic acid (9,12-octadecadienoic acid (Z, Z)-).

Among the total fatty acid content in earthworm extract, 69.9% were saturated, and 30.1% were unsaturated. In the cinnamon extract, 82.04% were saturated, and 17.96% were unsaturated. The most dominant saturated fatty acid in earthworm and cinnamon extracts was lauric acid (C12), a medium-chain fatty acid. Earthworms also contain unsaturated fatty acids, namely linoleic and oleic, and derivatives, while cinnamon contains only linoleic acid.

3.2. Phase 1 Clinical Trials

The results of the clinical symptoms observation are shown in Table 3. Based on the results, one volunteer on day 7 reported experiencing symptoms of bloating. On day 14, all volunteers showed no clinical symptoms. Based on Phase 1 clinical trials, the combination capsules showed very low clinical symptoms, with only 5% of volunteers experiencing mild symptoms in the first 7 days. This implies that the combination capsule can be considered safe for human treatment.

Shapiro-Wilk normality test showed that the initial cholesterol group data had a significance value (p) of 0.684 > 0.05, while the final cholesterol group data had 0.567 > 0.05. The initial blood sugar group data had a significance value (p) of 0.026 < 0.05, and the final blood sugar group data had 0.352 > 0.05.

Table 3. Clinical symptoms arising after consumption of combination capsules

Clinical symptoms	7 Days		14 Days	
	Number of subjects (n-20)			
	Yes	No	Yes	No
Nauseous	0	20	0	20
Bloating	1	19	0	20
Fatigue	0	20	0	20
Heartburn	0	20	0	20
Dizzy	0	20	0	20
Anorexia	0	20	0	20
Vomit	0	20	0	20

The significance value (p-value) for the Shapiro-Wilk normality test shows that both groups of cholesterol data are typically distributed. Subsequently, a paired t-test was conducted to determine the comparison of initial and final cholesterol. In both blood sugar groups, not all data were normally distributed. Wilcoxon test was then used to determine the initial and final sugar level difference.

The results in Table 4 show that the significance value was 0.036 (<0.05). Hence, H_0 was rejected. This result indicates that the initial and final cholesterol levels differed. Table 5 shows that the significance value was 0.003 (<0.05). Hence, H_0 was rejected, and H_1 was accepted. This hypothesis implies that the initial and final blood sugar levels differed positively. There was a significant difference between volunteers' cholesterol and blood sugar levels before and after the

Table 4. Comparison of initial and final cholesterol levels after 14-day treatment with earthworm-cinnamon capsules in phase 1 clinical trial (n = 20)

Paired samples test				
Parameter	Mean difference	t	df	p-value
Initial Cholesterol-Final Cholesterol	-11.75±5.20772†	-2.256	19	0.036*

Table 5. Comparison of initial and final blood sugar levels after 14-day treatment with earthworm-cinnamon capsules in phase 1 clinical trial (n = 20)

Rank				
		N	Mean rank	Sum of ranks
B-A	Negative ranks	7[a]	12.07	84.50
	Positive ranks	12[b]	8.79	105.50
	Ties	1[c]		
	Total	20		

a. B < A
b. B > A
c. B = A

Test statistics	
	Final blood sugar-initial blood sugar
Z	-.423[b]
Asymp. Sig. (2-tailed)	.003

a. Wilcoxon signed ranks test
b. Based on negative ranks

consumption of earthworm and cinnamon combined capsules.

3.3. Phase 2 Clinical Trials

Based on Figure 1, there was a decrease in the average cholesterol levels of women aged 21-30 years rom day 0 to day 14 by 241 mg/dl to 177 mg/ dl with a percentage of 25.56%. Among older women aged 61-70 years, cholesterol levels reduced from 318 to 276 mg/dl with a percentage of 13.21%. In men, the average cholesterol levels are shown in Figure 2. Among men aged 31-40, cholesterol reduced from 234 to 209 mg/dl with a percentage of 10.68%. For older men aged between 61-70 years, cholesterol levels reduced from 242 to 190.6 mg/dl with a percentage of 21.24%.

In Figure 3, blood sugar levels in women aged 21-30 years decreased from 201.6 to 161.3 mg/dl with a percentage of 19.99%. While in older women aged 61-70 years blood sugar reduced from 291 to 185 mg/dL with a percentage of 36.43%. In men, the average blood sugar levels are shown in Figure 4. Among men aged 31-40, blood sugar reduced from 281 to 180 mg/dl with a percentage of 35.94%. For older men aged between 61-70 years, blood sugar reduced from 283 to 173 mg/dl with a percentage of 38.87%.

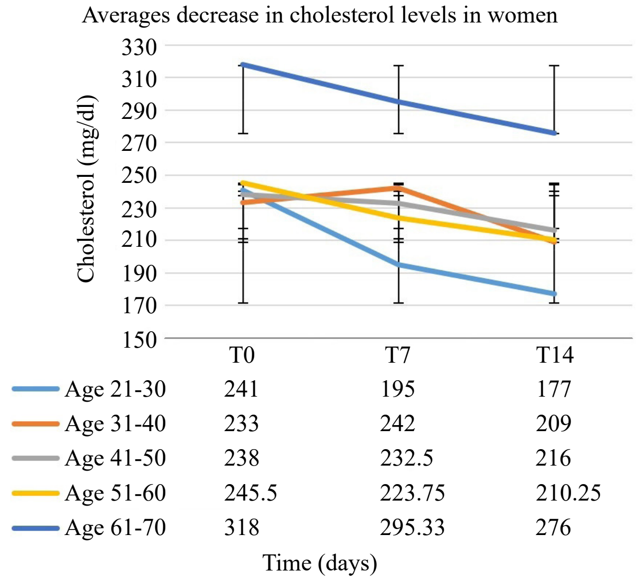


Figure 1. Graph of average cholesterol reductions in women

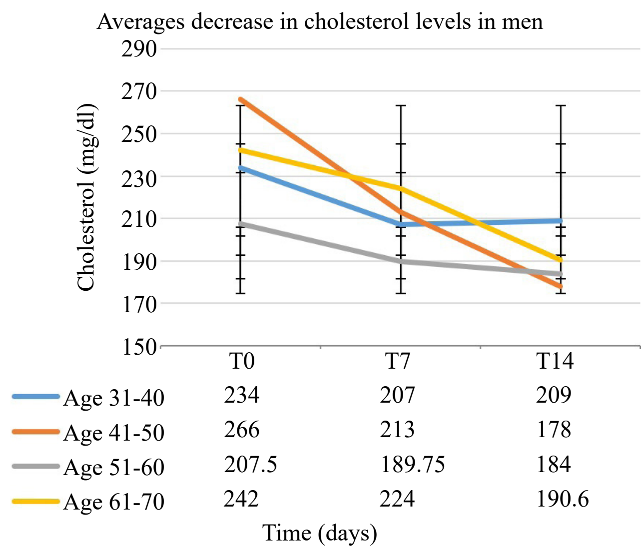


Figure 2. Graph of average cholesterol reductions in men

As shown in Table 6, the average decrease in cholesterol levels in men was more significant than in women, at 40.11 mg/dl and 36.27 mg/dl, respectively. Similarly, the average decrease in blood sugar levels in men was more significant than in women, at 63.50 and 54.00 mg/dl, respectively. According to Table 7, the most significant decrease in cholesterol levels (64.00 mg/dl) was found in the age range of 21-30 years, while the lowest decrease (29.37 mg/dl) was shown in the age range of 51-60 years. Regarding blood sugar levels, the most significant decrease (108.00 mg/dl) was found in the age range 61-70 years, while the lowest (40.30 mg/dl) was found in the age range 21-30 years.

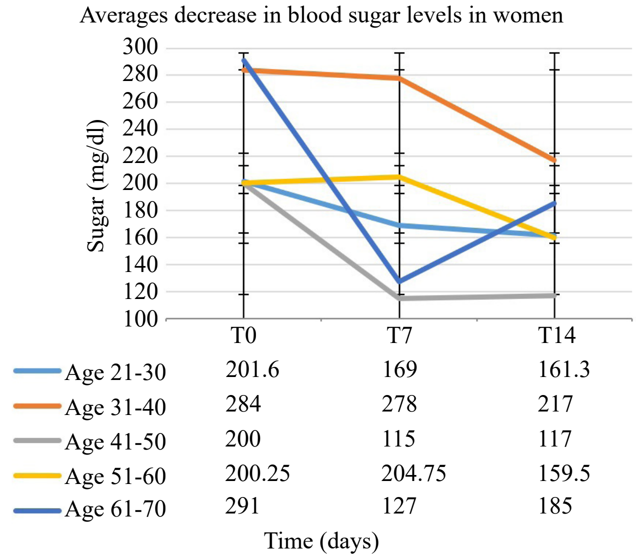


Figure 3. Graph of average blood sugar reductions in women

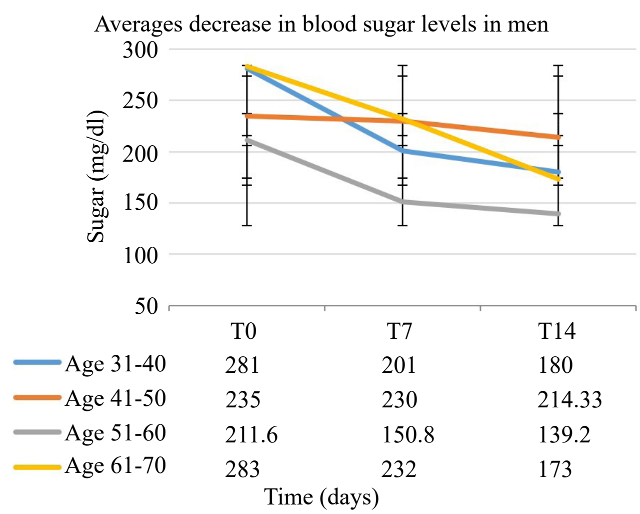


Figure 4. Graph of average blood sugar reductions in men

The normality test results showed that Groups A (T0), B (T7), and C (T14) data had significance values of 0.296 (>0.05), 0.031 (<0.05), and 0.015 (<0.05), respectively. Additionally, the normality test results for Group A (T0) showed a significance value of 0.340 (>0.05), Group B (T7) had a significance value of 0.068 (>0.05), and Group C (T14) had a significance value of 0.886 (>0.05).

The effect size (r) obtained from Wilcoxon and paired t-test was used in power analysis to determine sample size (n). More specifically, the effect size (r) in cholesterol levels was calculated by dividing the absolute (positive) standardized test statistic (Z) by the square root of the number of pairs (N) in the Wilcoxon test result in Table 8.

Table 6. Average decrease of cholesterol and blood sugar levels after 14-day treatment with earthworm-cinnamon capsules in phase 2 clinical trial (n = 20) based on gender

Gender	Average (mg/dl)					
	Initial cholesterol	Final cholesterol	Decrease	Initial blood sugar	Final blood sugar	Decrease
Women	262.36	226.09	36.27	218.10	164.10	54.00
Men	228.44	188.33	40.11	232.70	169.20	63.50

Table 7. Average decrease of cholesterol and blood sugar levels after 14-day treatment with earthworm-cinnamon capsules in phase 2 clinical trial (n = 20) based on age range

Age range (years)	Average (mg/dl)					
	Initial cholesterol	Final cholesterol	Decrease	Initial blood sugar	Final blood sugar	Decrease
21-30	241.00	177.00	64.00	201.60	161.30	40.30
31-40	233.50	193.50	40.00	282.50	198.50	84.00
41-50	252.00	197.00	55.00	217.50	165.66	51.83
51-60	226.50	197.12	29.37	205.92	149.35	56.57
61-70	280.00	233.30	46.70	287.00	179.00	108.00

Table 8. Comparison of initial and final cholesterol levels after 14-day treatment with earthworm-cinnamon capsules in phase 2 clinical trial (n = 20)

Rank				
		N	Mean rank	Sum of ranks
B-A	Negative ranks	3[a]	3.50	10.50
	Positive ranks	17[b]	11.74	199.50
	Ties	0[c]		
	Total	20		

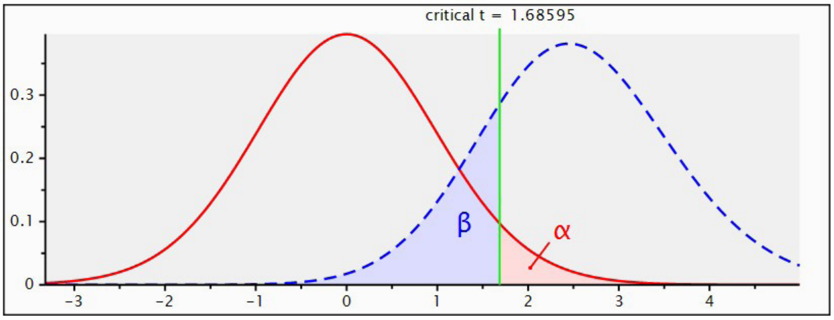
a. B < A
b. B > A
c. B = A

Test statistics	
	Final cholesterol-initial cholesterol
Z	-.3.529[b]
Asymp. Sig. (2-tailed)	<.001

a. Wilcoxon signed ranks test
b. Based on negative ranks

$$r = \frac{z}{\sqrt{N}} = \frac{3.529}{\sqrt{20}} = 0.789108$$

Power analysis (Figure 5) results showed that for sample size (n = 20) and alpha (α = 0.05), the actual power was 0.7897908 or 78%. This suggests a 78% probability of finding differences in the data. Meanwhile, Cohen's d value of the paired t-test presented in Table 9 corresponds to the effect size (r) in Blood Sugar Levels. With the r = 0.637, the result of power analysis (Figure 6) showed that for size (n = 20)



t tests - Means: Difference between two independent means (two groups)
Analysis:A priori: Compute required sample size

Input:	Tail(s)	=	One
	Effect size d	=	0.789108
	α err prob	=	0.05
	Power (1-β err prob)	=	0.78
	Allocation ratio N2/N1	=	1
Output:	Noncentrality parameter δ	=	2.4953786
	Critical t	=	1.6859545
	Df	=	38
	Sample size group 1	=	20
	Sample size group 2	=	20
	Total sample size	=	40
	Actual power	=	0.7897908

Figure 5. Power analysis for sample size (n) determination in cholesterol levels phase 2 clinical trial (n = 20)

Table 9. Samples effect size of initial and final blood sugar levels after 14-day treatment with earthworm-cinnamon capsules in phase I clinical trial (n =20)

Paired samples effect sizes						
			Standardize	Point estimate	95% confidence interval	
					Lower	Upper
Pair 1	Initial cholesterol-final cholesterol	Cohen's d	53.79775	-.637	-1.112	-.148
		Hedges' correction	56.04455	-.611	-1.067	-.142

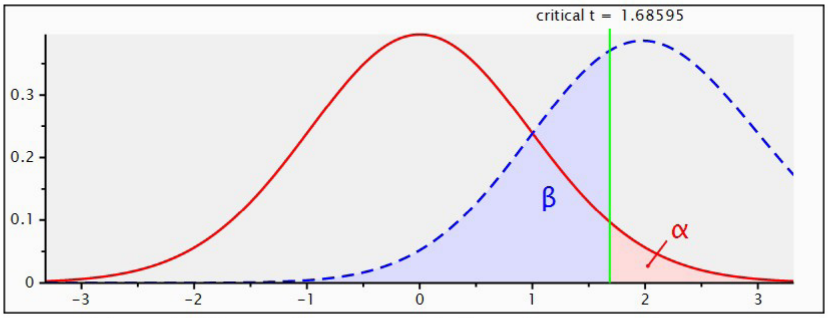
The denominator is used to estimate the effect sizes
Cohen's d uses the sample standard deviation of the mean difference
Hedges' correction uses the sample standard deviation of the mean difference plus a correction factor

and alpha (α = 0.05), the actual power was 0.6306035 or 63%.

4. Discussion

Data from measurement results in Phase 2 clinical trials were tested for significance values. Wilcoxon test results showed an Asymptotic Significance (2-tailed) value of <0.001, which is lower than the alpha value of 0.05. This indicates that H_0 was rejected, confirming a significant difference in cholesterol levels before and after taking a combination capsule of earthworm extract and cinnamon for 14 days. Cholesterol levels significantly declined after being treated with a combination capsule. The paired t-test on blood sugar data yielded a significance value of (0.010) shown in Table 10, less than 0.05. Consequently, H_0 was rejected, and H_a was accepted, confirming that the initial and the final blood sugar levels differed positively. In other words, there is a significant difference between volunteers' blood sugar levels before and after consuming the combination capsules.

The decrease in cholesterol levels was due to the content of fatty acids and active compounds, such as cinnamaldehyde, in the combination capsule of earthworm and cinnamon, which work synergistically. This combination contains saturated and unsaturated fatty acids. Saturated fatty acids, such as lauric, myristic, and stearic acid, can increase HDL levels by raising the secretion of apo A-1 from hepatocytes, transport rate of apo A1, HDL particle size, and decreasing the rate



t tests - Means: Difference between two independent means (two groups)
Analysis:A priori: Compute required sample size

Input:	Tail(s)	=	One
	Effect size d	=	0.637
	α err prob	=	0.05
	Power (1-β err prob)	=	0.63
	Allocation ratio N2/N1	=	1
Output:	Noncentrality parameter δ	=	2.0143709
	Critical t	=	1.6859545
	Df	=	38
	Sample size group 1	=	20
	Sample size group 2	=	20
	Total sample size	=	40
	Actual power	=	0.6306035

Figure 6. Power analysis for sample size (n) determination in blood sugar levels phase 2 clinical trial (n = 20)

Table 10. Comparison of initial and final blood sugar levels after 14-day treatment with earthworm-cinnamon capsules in phase 2 clinical trial (n = 20)

Paired samples test				
Parameter	Mean difference	t	df	p-value
Initial blood sugar-final blood sugar	-34.25±12.02954†	-2.847	19	0.010*

*Significant at p<0.05
†Values are presented as mean ± standard error of the mean (SEM)
df: degrees of freedom

of catabolism of the apo A-1 fraction. The increase in the amount and rate of HDL transport is an adaptation mechanism to the amount of cholesterol in the blood (Sayekti and Rustanti 2014). According to Sartika (2008), the content of unsaturated fatty acids such as oleic and linoleic acid has a beneficial effect on cholesterol levels in the blood. Oleic acid can lower K-LDL and increase K-HDL levels. Linoleic acid plays a crucial role in fat transport and metabolism, immune function, and maintaining cell membrane function and integrity.

The combination capsule of earthworm with cinnamon caused a decrease in blood sugar levels. Cinnamon generally contains fatty acids such as lauric, oleic, and linoleic. Lauric acid metabolism produces ketone bodies that cause an increase in insulin secretion, reducing blood sugar levels. It also increases the secretion and activity of GLP-1 (Glucagon Like Peptide-1), which can stimulate or increase insulin secretion from the pancreas gland, has a protective effect on pancreatic β cells, and inhibits the secretion of glucagon hormone, thereby regulating blood sugar levels (Saftiri *et al.* 2022). According to Firdausi *et al.* (2017), oleic and linoleic acids have an anti-diabetic effect, reducing blood sugar levels by increasing insulin secretion and peripheral sensitivity. Both acids improve peripheral insulin sensitivity with the help of GLP-1, which can increase the effect of insulin on glucose absorption. Lipogenesis indicates that peripheral cells are sensitive to the presence of insulin. Hence, GLP-1 can increase peripheral insulin sensitivity.

Aside from fatty acid content, cinnamaldehyde in the combination capsule has a synergistic role by influencing cholesterol mycelization, leading to cholesterol binding by bile acids *in vitro*. This bond can remove excess cholesterol in the body because bile acids are channelled through the duodenum to the outside of the body with faeces (Muliani 2015). According to Landani and Kurniawaty (2018), cinnamaldehyde can increase glucose transport by GLUT 4 in adipose cells and skeletal muscles to significantly reduce blood glucose.

The decrease in blood sugar levels can be attributed to several factors, including age and diet. According to Komariah and Rahayu (2020), age is related to the physiology of old age. As the age extends beyond 45 years, the body functions also decrease, including the work of the hormone insulin, leading to high blood sugar levels. Fatmawati and Mustin (2017) stated that the age above 45 years causes the function of body organs to decrease, thereby reducing the ability of pancreatic β cells to produce insulin. In older individuals, there was a decrease in mitochondrial activity in muscle cells by 35% (Hasyim *et al.* 2022). Factors of irregular eating patterns, such as high consumption of carbohydrates, proteins, and fats, are also very influential.

The higher decrease in cholesterol and blood sugar levels in men than in women is similar to the results obtained in some studies. Al-Maqati *et al.* (2022) reported that women tend to have a higher chance of developing hypercholesterolemia than men. This difference may be attributed to variations in probability distributions by gender and correlation with cholesterol and blood sugar levels, which are influenced by food intake, diet, and lifestyle. Another assumption can be made based on the gonadal hormones in men and women. Endogenous testosterone found in males is related to the risk of type II diabetes. In a previous study, the onset risk of diabetes was increased in males with reduced testosterone (Chen and Dou 2014). People with diabetes had significantly improved cholesterol levels, low-density lipoprotein (LDL), and triglyceride (TG) but also considerably reduced HDL levels, with females having higher values than males. Diabetics are likely to have an abnormal lipid profile along with more than one parameter affected. Therefore, females are more likely to have high cholesterol and blood sugar levels than males (Tharu and Tsokos 2017).

Gender and age were emphasized in this study without eliminating the other factors. The association between gender and cholesterol and blood sugar levels is relative. However, the hormonal difference between males and females is related to regulating cholesterol and blood sugar levels. Regarding the age factor, cholesterol and blood sugar levels were elevated as the age increased. The most significant decrease was found in the younger age range, except for blood sugar levels. This phenomenon was assumed to be affected by other factors, including lifestyle, food intake, BMI, and psychological stress.

The elderly volunteers generally showed high cholesterol levels and a slight decrease. The highest initial cholesterol levels were found in the age range 61-70 years old, but the reduction was more significant than in the age range 51-60 years old, according to Feng *et al.* (2020), the age factor was positively associated with total cholesterol (TC) levels in ≤40 years old and negatively associated in ≥61 years old. The common factor affecting TC levels is an inactive lifestyle. Furthermore, age factor was positively associated with TG levels in those ≤40 and negatively associated in those ≥56 years old. Body Mass Index (BMI) significantly affects TG levels irrespective of age. The age factor was positively associated with LDL among those ≤60 and negatively associated among those aged ≥61. HDL level was affected by an irregular factor. Al-Maqati *et al.* (2022)state that TC, TG, LDL, and HDL levels accelerate with age.

Blood sugar levels have a different pattern from cholesterol in correlation with age factors. The highest initial blood sugar levels and the most significant decrease were observed among those aged 61-70. Meanwhile, the lowest initial blood sugar levels and reduction were recorded among volunteers 21-30 years old. A previous study mentioned that blood sugar levels are more likely to increase with age (Fikriana and Devy 2018). There was a negative association between the age factor and glucose effectiveness (GE), which regulates the decrease in blood sugar levels (Huang *et al.* 2023). Therefore, it can be assumed that external factors, including lifestyle, food intake, and diet, played a role in this study. Psychological stress, smoking habits, and potassium intake positively correlate with high blood sugar levels (Ridwanto *et al.* 2020). The older age groups appeared to have better discipline in regulating the external factors mentioned.

From the Wilcoxon test, the effect size (n) in cholesterol levels treated with the combination capsules was 0.789 and categorized as medium for two independent means (groups) according to the criteria of effect size by Jacob Cohen in 1988. Similarly, the paired t-test showed an effect size (r) of 0.637 for blood sugar levels treated with the combination capsules after 14 days, also categorized as medium.

Effect size (r) interprets the practical significance of the results. It is a fundamental parameter in Power Analysis, which uses probability theory to assess the truthfulness of the null hypothesis (H_0). This analysis determines the likelihood of obtaining results without the underlying effects (Ellis 2010). This study used power analysis to determine the sample size of each data group (Phase 2 cholesterol and blood sugar levels) according to the actual powers. Based on the results, Phase 2 cholesterol group data has an actual power of 0.78 or 78% for the 20 sample size. In comparison, blood sugar group data has an actual power of 0.6306035 or 63% for 20 samples. These results imply a 78% and 63% chance of finding differences in each data group.

Despite the significant results obtained, this study has some limitations, with the first being the sample size. According to Hedges and Rhoads (2009), the acceptable power in normative statistical practice is 0.8 or above, and a larger sample size is required to achieve this power. Specifically, cholesterol group data required a minimum of 21 sample size, while in blood sugar group data, a minimum of 32 sample size was required. This study could not enlarge the sample size beyond 20 due to the minimum resources, including costs, staff, and burden on schools. Secondly, monitoring the volunteer group living in separate regions was not easy. Many factors might influence the measurement results of cholesterol and blood sugar levels and tend to make for less homogeneous data.

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