IFSCC 2025 full paper (IFSCC2025-1421)

"Biotransformation of Rare Ginsenosides C-O and Compound-MC1 Using Lactobacillus: Enhancing Klotho Expression for Anti-Aging Effects"

JINWOO MIN* [1], HEECHEOL KANG[1]
[1]R&D CENTER, GFC LIFE SCIENCE, HWASEONG-SI, Korea, South

1. Introduction

Recent years have witnessed a surge of interest in the Klotho gene and protein, particularly in the context of anti-aging and skin aesthetics. Klotho, first identified in 1997, is now recognized as a pivotal anti-aging gene expressed in various tissues, including the skin[1]. Elevated Klotho expression is closely linked to lifespan extension and enhanced cellular antioxidative and anti-inflammatory capacities[2]. Mechanistically, Klotho protein inhibits insulin/IGF-1 signaling and activates FOXO transcription factors, enabling cells to resist oxidative stress and directly protecting against UVA and UVB-induced damage[3]. Especially, Klotho expression decreases by over 60% in aged skin, contributing to diminished skin defense, collagen loss, wrinkle formation, and reduced elasticity-hallmarks of skin aging[4].

Klotho's multifaceted role in skin health includes promoting cell regeneration and wound healing, stimulating collagen synthesis, inhibiting collagen degradation, reducing transepidermal water loss (TEWL), and exerting robust anti-inflammatory and antioxidant effects[5]. Clinical studies have shown that topical application of Klotho-containing formulations improves wrinkles, skin texture, photodamage, and skin thickness, underscoring its potential as a novel target for skin rejuvenation and aesthetic enhancement[4-5].

Ginsenosides, the primary bioactive compounds in Panax ginseng, have long been valued for their anti-aging and health-promoting properties[6-7]. Among these, minor ginsenosides-defined as saponins naturally present at extremely low concentrations (often less than 0.1%)-are of particular interest due to their potent pharmacological activities[8]. Ginsenoside C-O and Compound-MC1 are two such rare minor ginsenosides, found only in trace amounts in ginseng roots. Their scarcity and the difficulty of extraction have historically limited their research and practical application[9]

Despite their rarity, both Ginsenoside C-O and Compound-MC1 have demonstrated remarkable biological activities. Ginsenoside C-O has been reported to exert strong

antioxidative and anti-inflammatory effects, while Compound-MC1 has shown neuroprotective and cardioprotective properties, including the ability to improve mitochondrial function, activate AMPK signaling, reduce oxidative stress, and suppress inflammatory cytokine production in various disease models[10]. These effects suggest a broad therapeutic potential for these minor ginsenosides.

To overcome the challenge of their limited natural abundance, recent advances have enabled the bioconversion of major ginsenosides into these rare minor forms using microbial processes. Microorganisms such as lactic acid bacteria and yeast, which produce β-glucosidase, can efficiently convert abundant major ginsenosides into pharmacologically active minor ginsenosides bioconversion strategy allows for the scalable and sustainable production of Ginsenoside C-O and Compound-MC1, making it feasible to explore their therapeutic applications more broadly[11].

In the present study, we utilized microbial bioconversion to significantly increase the yield of Ginsenoside C-O and Compound-MC1 from ginseng extracts. We then investigated, for the first time, the direct effects of these rare minor ginsenosides on Klotho gene expression and their molecular mechanisms in skin cells. Our findings reveal that both compounds not only retain their established antioxidative and anti-inflammatory benefits but also directly upregulate Klotho expression, introducing a novel mechanism for skin anti-aging and rejuvenation.
Therefore, this research aims to elucidate the direct impact of ginseng-derived minor ginsenosides on Klotho, and to uncover the molecular pathways by which they contribute to skin health and aesthetics. By establishing a scalable method for obtaining these rare compounds and demonstrating their efficacy in modulating Klotho, our study highlights their promise as next-generation ingredients for skin rejuvenation and anti-aging interventions.

2. Materia ls and Methods

Bio-Conversion and Purification of Ginsenoside

Roots of three-year-old Panax ginseng C.A. Meyer cv. Geumpung were finely ground and extracted with 70% ethanol at a ratio of 1:10 (w/v) under reflux at 80°C for 3 hours. The extract was filtered, concentrated under reduced pressure, and freeze-dried to yield a crude saponin powder. The dried extract was subjected to Diaion HP-20 column chromatography, sequentially eluted with water, 30%, 50%, and 70% ethanol to obtain a saponin-rich fraction.
To produce the minor ginsenosides Ginsenoside C-O and Compound-MC1, the saponin-rich fraction (10% w/v) was suspended in MRS broth and inoculated with *Lactobacillus plantarum* strain GFC01**.** Fermentation was carried out statically at 37°C for 21 days. The progress of biotransformation and the yields of Ginsenoside C-O and Compound-MC1 were monitored by high-performance liquid chromatography (HPLC) using a C18 column and UV detection at 203 nm. After fermentation, the broth was subjected to medium-pressure liquid chromatography (MPLC) using a silica gel and C18 column, with a linear gradient of acetonitrile and water as

the mobile phase. Fractions containing high concentrations of Ginsenoside C-O and Compound-MC1 were pooled and lyophilized for subsequent experiments.

Cell culture

The human keratinocyte (HaCaT) and human fibroblast (HFF) cell line were cultured in Dulbecco's modified Eagle medium, supplemented with 10% fetal bovine serum and 1% of a penicillin–streptomycin solution. Cells were maintained at 37 ℃ with 5% CO2.

Western blot analysis

Equal amounts of protein were separated on SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride membranes. Transferred membranes were blocked and then incubated with primary antibodies: phospho-AKT (Ser473), total AKT, phospho-FoxO3a (Ser253), total FoxO3a, and Klotho. β-actin. Following washing with TBST, membranes were incubated with HRP-conjugated secondary antibody. The membranes were developed on a chemiluminescence imaging system

Statistical analysis

All experiments were performed in triplicate. Data are presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (SAS 9.4 software). Differences were considered statistically significant at $p < 0.05$.

3. Results

Bioconversion

The bioconversion of ginseng saponins using *Lactobacillus* was analyzed by HPLC with a C18 column at 203 nm. As shown in the table, the contents of major ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rg1) significantly decreased in the bioconversion group compared to the control group. In the control, the amounts of Rb1, Rb2, Rc, Rd, Re, and Rg1 were 3.16 mg/g, 2.18 mg/g, 2.25 mg/g, 1.54 mg/g, 1.84 mg/g, and 1.04 mg/g, respectively. After bioconversion, these values decreased to 0.15 mg/g, 0.33 mg/g, 0.14 mg/g, 0.18 mg/g, 0.26 mg/g, and 0.10, respectively.

In contrast, minor ginsenosides such as C-O, Mc1, C-Y, and C-K, which were not detected (ND) in the control group, were newly detected after bioconversion, with contents of 0.38 mg/g, 0.39 mg/g, 0.25 mg/g, and 0.97 mg/g, respectively. These results indicate that major ginsenosides were converted into minor ginsenosides through enzymatic hydrolysis by *Lactobacillus* during the bioconversion process. In particular, the content of C-K, known for its high pharmacological activity, increased to 0.97mg/g after bioconversion.

Additionally, Mc1 and C-O were purified using column chromatography, and their purity was confirmed to be over 98%. These highly purified compounds were subsequently used for biological activity tests.

Overall, these findings demonstrate that *Lactobacillus*-mediated bioconversion effectively changes the ginsenoside profile of ginseng by increasing the content of minor ginsenosides.

Table 1. A comparison of ginsenoside in ginseng before and after bioconversion

mg/g

	Rb1	Rb2	Rc	Rd	Re	Rg1	C-O	Mc1	C-Y	C-K
Control	3.16	2.18	2.25	1.54	1.84	1.04	ND	ND	ND	ND
Bio-Conversion	0.15	0.33	0.14	0.18	0.26	0.1	0.38	0.39	0.25	0.97

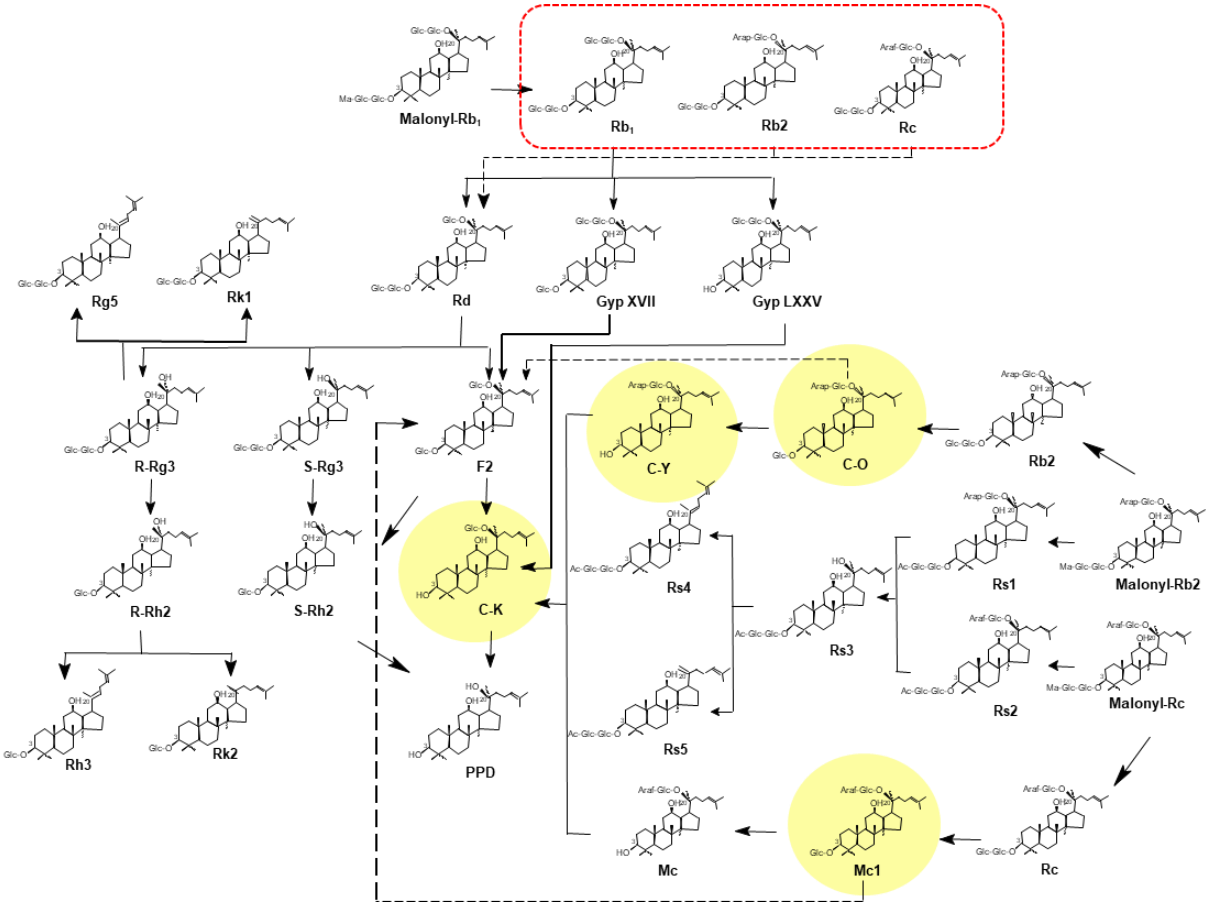

Figure 1. ginsenoside bioconversion pathway

Effects of Various Ginsenosides on Klotho Protein Expression

In this study, we investigated the effects of different ginsenosides on Klotho protein expression. Western blot analysis was conducted to compare natural ginsenosides commonly found in ginseng (Rb1, Rb2, Rd, Re, and Rg1) with bio-converted minor ginsenosides (C-O, MC1, F1, C-Y, and C-K). As shown in Figure 2, most ginsenosides enhanced Klotho protein expression compared to the control group. Especially, the bio-converted minor ginsenosides demonstrated significantly higher Klotho-inducing capacity than their natural counterparts. Among all tested compounds, compound C-O exhibited the most pronounced effect on Klotho protein upregulation. These findings align with previous research suggesting that rare saponins show higher pharmacological activity due to reduced sugar residues in their molecular structure, increased hydrophobicity, and enhanced cellular penetration. The upregulation of Klotho by ginsenosides may contribute to their renoprotective effects through inhibition of the PI3K/AKT pathway and subsequent activation of FoxO3a-mediated antioxidant mechanisms[12]. Our results confirm previous observations that multiple ginsenoside components can restore Klotho protein levels in models of cellular injury. This differential effect between natural and bio-converted ginsenosides provides valuable insights for developing more potent therapeutic agents targeting Klotho-mediated signaling pathways in various pathological conditions, particularly those associated with Klotho deficiency.

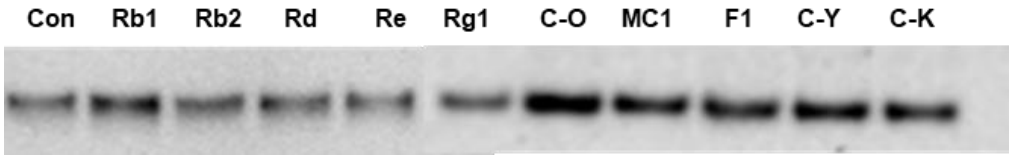

Figure 2. Effects of natural and bio-converted ginsenosides on Klotho protein expression.
Representative Western blot images showing Klotho protein levels in cells treated with natural ginsenosides (Rb1, Rb2, Rd, Re, Rg1) and bio-converted minor ginsenosides (C-O, MC1, F1, C-Y, C-K) compared to the control group;

Effects of MC-1 and C-O treatments on Klotho expression and PI3K/AKT/FOXO3 signaling in cells.

Based on the Western blot analysis shown in the image, our results demonstrate the effects of ginsenoside MC-1 and C-O treatments on Klotho expression and related signaling pathways. The Western blot data reveals that C-O treatment significantly upregulated Klotho protein expression compared to both control and MC-1 treated groups. This observation confirms the efficacy of C-O in enhancing Klotho protein levels.

Regarding the PI3K/AKT pathway, while PI3K expression remained relatively consistent across treatment groups, phosphorylated AKT (p-AKT) levels were especially reduced in both MC-1 and C-O treated groups compared to control, with a more pronounced decrease in the C-O group. Total AKT (t-AKT) expression remained stable across all conditions, indicating that the observed changes were specific to the phosphorylation state rather than total protein abundance. These findings align with the known function of Klotho as a negative regulator of the PI3K/AKT pathway.

The phosphorylated FOXO3 (p-FOXO3) expression was minimally detected across all treatment groups, suggesting inhibition of FOXO3 phosphorylation. This reduction in FOXO3 phosphorylation is consistent with decreased p-AKT activity and likely promotes nuclear accumulation of FOXO3, as non-phosphorylated FOXO3 can translocate to the nucleus. Total FOXO3 (t-FOXO3) levels showed no significant differences between groups.

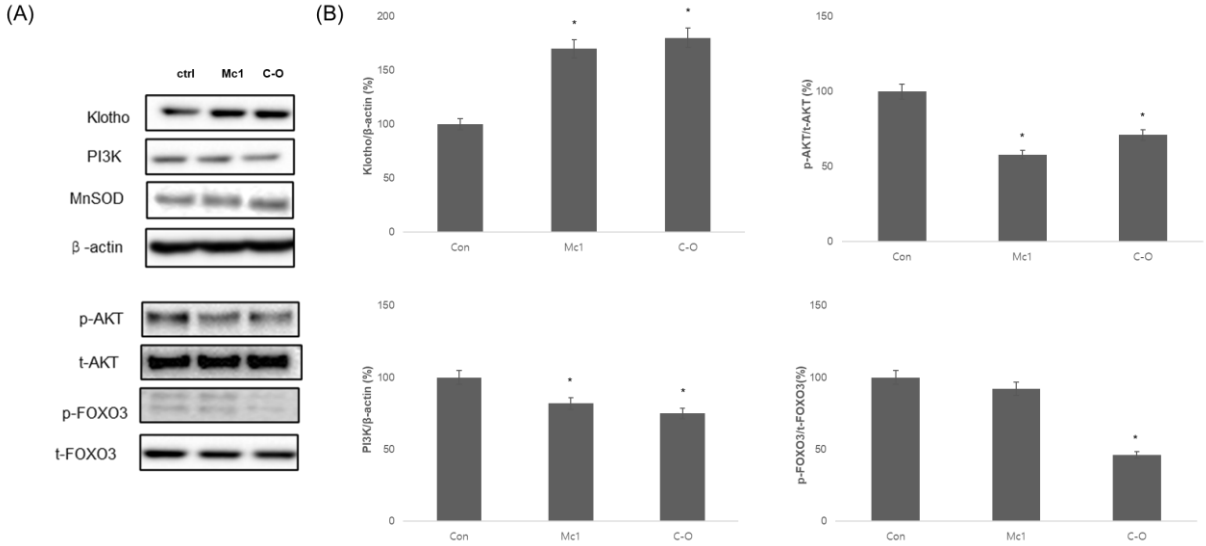

Figure 3. Effects of MC-1 and C-O treatments on Klotho expression and PI3K/AKT/FOXO3 signaling in cells.

(a) Representative Western blot images showing protein expression (b) Quantitative analysis of Western blot results. Bar graphs display the relative protein expression ratios for Klotho/β-actin, p-AKT/t-AKT, PI3K/β-actin, and p-FOXO3/t-FOXO3 (% of control). Data are presented as mean ± SEM. *p < 0.05 vs. control.

Effects of MC-1 and C-O Treatments on AMPK and NF-κB Signaling Pathways in Cells

The Western blot results show(Figure 4) that both bio-conversion-derived ginsenosides MC-1 and C-O significantly increased the phosphorylation of AMPK (P-AMPK) compared to the control group, while the total AMPK and β-actin levels remained consistent across all groups. Specifically, the intensity of the P-AMPK band is noticeably higher in both the MC-1 and C-O groups than in the control, indicating that these ginsenosides effectively activate AMPK signaling.

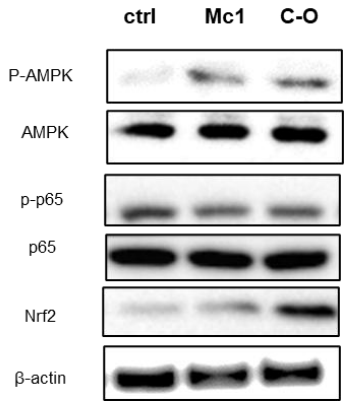

Figure 4. Effects of MC-1 and C-O treatments on AMPK, NF-κB, and Nrf2 signaling pathways in cells. Representative Western blot images showing the protein expression levels of phosphorylated AMPK (P-AMPK), total AMPK, phosphorylated p65 (p-p65), total p65, Nrf2, and β-actin in control (ctrl), MC-1 (Mc1), and C-O treatment groups.

This selective increase in P-AMPK, without changes in total AMPK, demonstrates that MC-1 and C-O promote AMPK activation rather than altering its overall protein expression. Since Furthermore, the results show that phosphorylated p65 (p-p65) levels were decreased by MC-1 and C-O treatment, indicating inhibition of the NF-κB signaling pathway, which is closely associated with inflammatory responses. This reduction in p-p65 suggests that MC-1 and C-O may exert anti-inflammatory effects by suppressing the expression of inflammation-related genes. In contrast, Nrf2 levels were increased, implying an upregulation of antioxidant gene expression and enhanced cellular protection against oxidative stress.

Collectively, these findings demonstrate that MC-1 and C-O simultaneously activate AMPK, suppress NF-κB-mediated inflammation (as indicated by decreased p-p65), and enhance antioxidant defense mechanisms (as indicated by increased Nrf2). These molecular changes strengthen the cellular anti-inflammatory and antioxidant defense systems, highlighting the potential of MC-1 and C-O as promising candidates for anti-aging and antioxidant cosmetic ingredients.

4. Discussion

Based on the Western blot analyses, both bio-conversion-derived ginsenosides MC-1 and C-O significantly increased Klotho protein expression compared to the control group (Figure 3a, 3b). This upregulation of Klotho, a well-known anti-aging and antioxidative protein, is consistent with previous findings that ginsenosides can modulate Klotho expression and contribute to anti-aging effects.

In the PI3K/AKT pathway, PI3K levels remained relatively unchanged among the groups, while phosphorylated AKT (p-AKT) was markedly decreased in both MC-1 and C-O treated groups. Total AKT (t-AKT) levels were consistent, indicating that the observed changes were specific

to the phosphorylation state (Figure 3a, 3b). This result supports the notion that both ginsenosides suppress AKT activation, likely via Klotho upregulation, which is known to negatively regulate the PI3K/AKT pathway[1]. Ginsenosides have been reported to modulate PI3K/AKT signaling, contributing to antioxidative and cytoprotective effects[8].

For FOXO3 signaling, phosphorylated FOXO3 (p-FOXO3) levels were reduced in both MC-1 and C-O groups, while total FOXO3 (t-FOXO3) expression remained unchanged (Figure 3a, 3b). The decrease in p-FOXO3 is consistent with reduced AKT activity and may facilitate the nuclear localization and activation of FOXO3, leading to enhanced expression of antioxidant genes[12].

Additionally, analysis of the AMPK pathway revealed that phosphorylated AMPK (p-AMPK) levels were increased in both MC-1 and C-O treated groups compared to control, while total AMPK levels were unchanged (Figure 4). Activation of AMPK is associated with improved cellular energy homeostasis and stress resistance, and ginsenosides have been shown to activate AMPK, contributing to their beneficial effects on cellular metabolism and anti-aging[13-14].

Collectively, these results demonstrate that both bio-conversion-derived ginsenosides MC-1 and C-O upregulate Klotho expression, inhibit the PI3K/AKT pathway, promote FOXO3 activation, and enhance AMPK signaling. These coordinated effects contribute to improved antioxidant defense, cellular energy balance, and anti-aging potential, supporting the application of bio-conversion ginsenosides as promising active ingredients in premium anti-aging cosmetic formulations

5. Conclusion

Ginsenosides MC-1 and C-O, produced through *Lactobacillus*-mediated bioconversion, significantly increased the expression of the anti-aging protein Klotho in cell experiments. Both compounds inhibited the phosphorylation of AKT in the PI3K/AKT signaling pathway and reduced the phosphorylation of FOXO3, suggesting the potential to activate antioxidant gene expression. In addition, both MC-1 and C-O markedly increased AMPK phosphorylation, which contributes to improved cellular energy homeostasis and enhanced resistance to stress. These results indicate that bioconverted ginsenosides MC-1 and C-O can enhance antioxidant defenses and anti-aging effects, supporting their strong potential as premium active ingredients for anti-aging cosmetic formulations.

6. Reference

[1] Kuro-o M, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature. 1997;390(6655):45-51.

[2]Cognitive Vitality, Alzheimer's Drug Discovery Foundation. Klotho (Target). 2021.

[3] Yamamoto M, et al. Regulation of oxidative stress by the anti-aging hormone klotho. J Biol Chem.

[4] Dalton GD, et al. Pathobiology of the Klotho Antiaging Protein and Therapeutic Considerations. Front Aging. 2022;2:931331.

[5] Prud'homme GJ, et al. Pathobiology of the Klotho antiaging protein and therapeutic considerations. Front Aging. 2022;2:931331.

[6] Li X, et al. Effect of anti-skin disorders of ginsenosides-A systematic review. Phytomedicine Plus. 2023;3(2):100404.

[7] Kim JH, et al. Ginseng and ginseng byproducts for skincare and skin health. J Ginseng Res. 2024;48(5):569-583.

[8] Chen S, et al. Research progress on anti-aging effects and mechanisms of the rare ginsenoside Compound K. J Ginseng Res. 2022;46(5):711-720.

[9] Zhang Y, et al. β-Glucosidase and its application in bioconversion of ginsenosides. Molecules. 2023;28(8):3572

[10] Liu Y, et al. Progress on anti-aging effects and mechanisms of the new ginsenoside Compound K. J Dermatol Sci Cosmet Technol. 2023;2(1):1-10.

[11] Geraldi A, et al. Advances in the production of minor ginsenosides using biological methods. Bio-Integration. 2020;1(2):53-61.

[12] Yamamoto M, et al. Regulation of oxidative stress by the anti-aging hormone klotho. J Biol Chem. 2005;280(45):38029-34.

[13] Kim JH, et al. Ginseng and ginseng byproducts for skincare and skin health. J Ginseng Res. 2024;48(5):569-583.

[14] Li X, et al. Effect of anti-skin disorders of ginsenosides-A systematic review. Phytomedicine Plus. 2023;3(2):100404.