Mechanism of action of Indonesian medicinal plants in inhibiting 3T3-L1 adipocyte differentiation: A review

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ABSTRACT
Obesity is one of the most serious health problems worldwide, including in Indonesia. One of the strategies to inhibit obesity is modulating adipogenesis, as obesity is associated with the dysregulation of adipogenesis. Adipogenesis is mainly influenced by peroxisome proliferator-activated receptor gamma and CCAAT/enhancer-binding protein alpha; hence, substances that can inhibit the expression of both transcription factors can be key in obesity management. This review aims to elaborate upon Indonesian medicinal plants that can inhibit adipogenesis in 3T3-L1 adipocytes and their mechanism of action. Original research articles published in 2016–2020 and obtained from PubMed and CINAHL databases were included in this study. From 226 articles, 5 Indonesian medicinal plants (Catharanthus roseus, Chromolaena odorata, Lagerstroemia speciosa, Oroxylum indicum, and Spiranthes sinensis) have been identified to have an inhibitory effect on 3T3-L1 adipogenesis, by suppressing the induction of Pparg, Cebpa, and other adipogenic and lipid metabolism-related genes. Further research is required to identify the responsible chemical compounds that yield this effect and to elaborate on the mechanisms by which these compounds inhibit the induction of adipogenic genes.

INTRODUCTION
Obesity is a condition characterized by increased body mass index (BMI) (Hruby and Hu, 2015). Based on the Asia-Pacific Task Force, obesity is defined as BMI ≥ 25 kg/m² (Lim et al., 2017). The underlying causes of obesity are complex and multifactorial. Overweight and obesity affect one-third of the world’s population (Lim et al., 2017). Obesity is considered one of the most serious health problems because it is one of the main risk factors for chronic diseases, such as cardio- and cerebrovascular diseases, diabetes mellitus type 2, and cancer (Hruby and Hu, 2015).

The prevalence of obesity continues to increase worldwide. It is a major health problem in developing countries that experience economic growth such as the Southeast Asia regions, including Indonesia. It causes a transition in health from nutritional deficiency to excess nutrition that triggers obesity (World Health Organization, 2022). The prevalence of obesity in Indonesia is very high. In 2018, the prevalence of obesity in Indonesia was approximately 35.4% (Riskesdas, 2018). This prevalence increased from 26.6% in 2013 (Riskesdas, 2013). If it is not treated immediately, obesity can cause an economic burden, both due to health costs resulting from obesity complications and decreased work productivity.

Previous studies revealed that the number of adipocytes, which can be measured by adipocyte turnover analysis, in the human body is determined since childhood and, in normal
conditions, tends to be constant until adulthood (Arner, 2018). However, the number of adipocytes can increase (hyperplasia) when someone has a history of obesity before 5 years old or has a lifestyle of consuming excess calories as an adult (Arner, 2018). Adipocyte hyperplasia and excess energy state will cause fat accumulation, which will trigger hypertrophy of adipocytes (Arner, 2018; Kahn et al., 2019). Hyperplasia of adipocytes during adipogenesis is considered the pathogenesis of obesity (Lessard and Tchernof, 2012).

The 3T3-L1 preadipocyte cell line is often used to study the transcription process in adipocyte differentiation, such as the expression of CCAAT/enhancer-binding protein (C/EBP) and peroxisome proliferator-activated receptor (PPAR) to promote adipogenesis. The expression of these genes causes an increase in glucose uptake and triglyceride synthesis. As a result, the cells will begin to show signs of lipid accumulation four days after being exposed to the differentiation medium (Ali et al., 2013; Zebisch et al., 2012). Adipogenesis is influenced by various internal and external factors. Phytochemical content or components of medicinal plants are thought to have various inhibitory effects in the adipogenesis process (Chang and Kim, 2019).

Traditional medicine derived from medicinal plants has been known for a long time, especially in eastern countries, to treat various health problems including obesity. This is due to its low costs, empirical effectiveness, and low side effects. Advances in technology and medical sciences widen the opportunity to elaborate on the effects of medicinal plants on human health and to identify the responsible active compounds for the beneficial effects. It can, in turn, be utilized for disease prevention and treatment. Several substances derived from traditional medicine that is already known to have anti-obesity effects are artemisinin, curcumin, celandrol, capsaicin, berberine, and ginsenosides (Xu et al., 2018).

Indonesia is one of the countries with the largest biodiversity worldwide as indicated by the presence of 80% of medicinal plants located throughout the world. Historically, at least 6,000 herbal plants have been used by Indonesians as traditional medicine (Elfahmi et al., 2014). Traditional medicine based on medicinal herbs is essential in modern medical research, especially for countries with eastern cultures such as Indonesia. This is due to a large number of medicinal plants used as traditional medicine in Indonesia and the phytochemical potential of medicinal plants which is not fully understood yet. Therefore, research on medicinal plants needs to be carried out to determine their bioactivity potential.

One of the potentials utilized from medicinal plants is the potential for anti-obesity (Liu et al., 2017). Some medicinal plants in Indonesia are found to treat or prevent obesity, due to their ability to inhibit the adipogenesis process from preadipocyte to adipocyte as shown in 3T3-L1 cells. This review aims to elaborate on the mechanism by which Indonesian medicinal plants inhibit adipogenesis in the 3T3-L1 cell line. We hope that this review can provide valuable information regarding the effects of medicinal plants both in Indonesia and in the world in reducing obesity rates worldwide.

This review analyzed works of literature obtained from PubMed and CINAHL using MeSH keywords with a strategy: (plants, medicinal OR Asia OR Southeast Asia OR Indonesia) AND (Adipogenesis OR Adipocyte OR Adipocyte, White) AND 3T3-L1 Cells. The literature search was conducted from August to December 2020.

The inclusion criteria in the study were original research articles that described the mechanism of the Indonesian medicinal plants on adipogenesis in 3T3-L1 cell lines and were published in the last 5 years (January 2016–December 2020). Articles whose full text or abstract could not be accessed, articles in languages other than Indonesian or English, and articles that did not mention Indonesian medicinal plants or 3T3-L1 cell lines were excluded. To ascertain whether the plants’ endemic habitat included Indonesia, we used https://www.gbif.org/.

The research procedure was described as follows (Fig. 1): (1) Literature search is based on scientific articles using the strategy described above; (2) Remove duplicates if the same article title is found by looking at the title; (3) Screen the literature by looking at the title and abstract. Titles and abstracts that meet the exclusion criteria will be discarded; (4) Conduct literature eligibility by looking at the abstract and full text of each piece of literature. The literature that does not meet the inclusion criteria will be discarded; and (5) Perform analysis and synthesis of the literature that meets the inclusion criteria. The literature search process was carried out (Fig. 1) and 226 articles were found on electronic search engines: PubMed (n = 163) and CINAHL (n = 63). Based on the established inclusion and exclusion criteria, six articles were identified that discussed the relationship between Indonesian medicinal plants and the adipogenesis process in the 3T3-L1 cell line. From these six articles, we identified five Indonesian medicinal plants that inhibit adipogenesis in 3T3-L1 cells: Catharanthus roseus, Chromolaena odorata, Lagerstroemia speciosa, Oroxylum indicum, and Spiranthes sinensis (Table 1, Fig. 2).

**INDONESIAN MEDICINAL PLANTS THAT INHIBIT 3T3-L1 ADIPOCYTE DIFFERENTIATION**

*Catharanthus roseus*

*Catharanthus roseus* (Family: Apocynaceae), known as *Tapak dara* or *Kembang sari Cina* in Indonesia, is a plant often found throughout the world. It is well-known as an ornamental plant and can be found in all regions of Indonesia. This plant is most commonly found in Java, Sulawesi, Kalimantan, and Sumatra islands. It has various benefits, especially as a medicinal plant, where various properties were identified (Borah et al., 2019; Nejat et al., 2015). *Catharanthus roseus* is known to have anti-diabetic, anti-microbial, anti-mutagenic, anti-mitotic, antioxidant, and anti-cancer effects (Nejat et al., 2015). It contains many alkaloids and phenolics, which are the main components of this plant, but others are also found, such as alkaloids, flavonoid glycosides, polyphenols, anthocyanins, steroids, and iridoid glycosides (Nejat et al., 2015). Based on research from Borah et al. (2019) the extract from the leaves of the plant can inhibit the adipogenesis process in 3T3-L1 cells.

*Catharanthus roseus* leaf extract was able to inhibit the expression of PPARγ1 and PPARγ2 messenger-RNA and protein. This was evidenced by the decreased levels of adipocyte markers affected by PPARγ, such as perilipin 1 (PLN1), fatty acid binding protein 4 (FABP4), lipoprotein lipase (LPL), adipose triglyceride
lipase (ATGL), glucose transporter 4 (GLUT4), and adiponectin (Borah et al., 2019). Various transcription factors, both stimulatory and inhibitory for the adipogenesis process, were influenced by leaf extracts. In the early stages of differentiation, there was a decrease in the level of stimulatory Lipin1 and an increase in the level of the inhibitory Kruppel-like factor (KLF7). Meanwhile, at the final stage of differentiation, there was a decrease in levels of C/EBPα, Lipin1, and other similar positive effectors such as fatty acid synthase (FAS) and sterol regulatory element binding protein 1c (SREBP1c) in the extract-treated cells (Borah et al., 2019).

Table 1. Mechanism of action by which Indonesian medicinal plants inhibit adipogenesis in the 3T3-L1 cell line.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Part used</th>
<th>Phytochemical compound</th>
<th>Mechanism of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catharanthus roseus</td>
<td>Leaves</td>
<td>1α, 25-Dihydroxy vitamin D3</td>
<td>↓ Transcription factors [C/EBPα, Lipin1, PPARγ1 and PPARγ2, and SREBP-1c] ↑ Transcription factor (KLF7) ↑ Adipogenic specific genes (PLN1, FAS, FABP4, LPL, ATGL, GLUT4, and adiponectin)</td>
<td>Borah et al. (2019)</td>
</tr>
<tr>
<td>Chromolaena odorata</td>
<td>Leaves</td>
<td>Kaempferide and DTMC</td>
<td>↓ Transcription factor (PPARγ) ↓ Mitotic clonal expansion ↓ Transcription factors (C/EBPα and PPARγ) ↑ Adipogenic specific gene (adiponectin)</td>
<td>Kumkarnjana et al. (2019)</td>
</tr>
<tr>
<td>Lagerstroemia speciosa</td>
<td>Leaves</td>
<td>DLBS3733</td>
<td>↓ Transcription factors (C/EBPα and PPARγ) ↑ Adipogenic specific gene (adiponectin)</td>
<td>Karsono et al. (2019)</td>
</tr>
<tr>
<td>Oroxylum indicum</td>
<td>Fruits</td>
<td>Quercetin, apigenin, and baicalein (hypothesized)</td>
<td>↓ Transcription factors (PPARγ2 and SREBP-1c) ↑ Adipogenic specific genes (FAS, GLUT4, and leptin)</td>
<td>Hengprathom et al. (2020)</td>
</tr>
<tr>
<td></td>
<td>Root barks</td>
<td>Oroxylin A, chrysins, and baicalein</td>
<td>↓ Transcription factors (C/EBPα and PPARγ) ↑ Adipogenic specific genes (C/EBPα and SREBP-1c)</td>
<td>Mangal et al. (2017)</td>
</tr>
<tr>
<td>Spiranthes sinensis</td>
<td>Whole plants</td>
<td>Sinensol-C</td>
<td>↓ Transcription factors (PPARγ, C/EBPα, and SREBP-1c) ↑ Adipogenic specific genes (FAS and FABP4)</td>
<td>Shie et al. (2020)</td>
</tr>
</tbody>
</table>
The molecule that can inhibit the adipogenesis process in the leaves was 1α, 25-dihydroxy vitamin D3. The content of 100 nM (41.6 ng/ml) 1α, 25-dihydroxy vitamin D3 in C. roseus leaves can inhibit adipocyte maturation and differentiation. Therefore, 1α, 25-dihydroxy vitamin D3 can be used as a potential active substance as an anti-obesity drug (Borah et al., 2019).

Chromolaena odorata

Chromolaena odorata, known as Minjangan grass or kirinyuh in Indonesia, is a medicinal plant found in all regions of Indonesia and is sometimes used as an ingredient in herbal medicine (Padmanaba et al., 2017; Vijayaraghavan et al., 2017). This plant belongs to the family Compositae (Vijayaraghavan et al., 2017). Chromolaena odorata or kirinyuh has been reported to have anti-bacterial, anti-plasmodic, anti-protozoal, anti-trypanosomal, anti-fungal, anti-hypertensive, anti-inflammatory, immunomodulatory, diuretic, hepatotropic, anti-cancer, and astringent effects. Chemical analysis of this plant has been carried out to identify substances including monoterpenes, hydrocarbon sesquiterpenes, triterpenes/steroids, alkaloids, and flavonoids (Vijayaraghavan et al., 2017). The leaves are rich in flavonoids, including sinensetin, padmatine, quercetin, kaempferol, sakuranetin, and salvigenin. Its extract is also known to have flavonoids (sakuranetin, salvigenin, kaempferide, isosakuranetin, betulenol, tamarixetin, 2-5-7-3 tetra-o-methyl quercetagetin, two chalcone and odoratin, and other alcohol components), essential oils (β-eubeden, geyren, and bornyl acetate), triterpenoid, tannins, saponins, organic acids, and various other small substances (Vijayaraghavan et al., 2017).

In the research conducted by Kumkarnjana et al. (2019), C. odorata leaves containing flavonoids inhibited the adipogenesis process in the 3T3-L1 cell line. Kaempferide and 4,20-dihydroxy-40,50,60-trimethoxychalcone (DTMC) in the leaves were able to reduce the level of PPARγ gene expression. Kaempferide is a derivative of kaempferol, which only has differences in the hydroxyl group in ring B of the kaempferol with a methoxy group, whereas DTMC is classified as a chalcone (Kumkarnjana et al., 2019). When comparing the administration of DTMC and kaempferide in the early phase of differentiation (days 0–3) with the late phase of differentiation (days 3–9), almost the same effect was observed. Therefore, DTMC and kaempferide are thought to act in the early stages of differentiation by inhibiting mitotic clonal expansion (Kumkarnjana et al., 2019).

Lagerstroemia speciosa

Lagerstroemia speciosa L, or bungur in Indonesia, is a medicinal plant native to Southeast Asia. It is found in all regions of Indonesia, except Maluku. This plant belongs to the Lythraceae family and is known for its attractive appearance (Nurcahyanti et al., 2018). Bungur as traditional medicine has been known for the treatment of diabetes, obesity, and kidney disease (Rohit Singh and Ezhilarasan, 2020). This plant is known to have various pharmacological properties such as anti-diabetic, anti-obesity, anti-viral, anti-bacterial, cytotoxic, anti-inflammatory, and anti-nociceptive activities (Koduru et al., 2017). More than 40 phytochemicals can be found in the leaves, such as ellagic acid and its derivatives, tannins, triterpenes, isoquercetin, triterpenoid, quercetin, corosolic acid, flavones, and glycosides (Mousa et al., 2019). The fraction of the leaves, namely DLBS3733, which came from an aqueous extract of L. speciosa leaves, can inhibit the adipogenesis process in the 3T3-L1 cell line. Reduction in the levels of PPARγ, C/EBPα, and adiponectin indicated the anti-adipogenic effect of DLBS3733 at doses of 7.5 and 15 µg/ml, in a dose-dependent manner (Karsono et al., 2019). As mentioned earlier, adiponectin expression is strongly influenced by PPARγ (Astapova and Leff, 2012). The ability of DLBS3733, derived from L. speciosa leaf extract, to inhibit the adipogenesis process could be a potential target for obesity management.

Oroxylum indicum

Oroxylum indicum or bungli is a medicinal plant that can grow to a height of 12 m and often branches irregularly. This plant belongs to the Bignoniaceae family and can be found in India, Sri

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**Figure 2.** Five Indonesian medicinal plants that inhibit adipogenesis in 3T3-L1 cells.
Lanka, Southeast Asia, the Philippines, and Indonesia. This plant is found in Sumatra, Java, Kalimantan, and the Sulawesi islands. It is often planted near the community yard (Karnati et al., 2013; Rasadah, 2001). The leaves, bark, root bark, fruit, and seeds of this plant have medical benefits, are often used in traditional medicine, and have different benefits from location to location. This is due to the various chemical constituents of O. indicum such as flavonoids, glycosides, alkaloids, tannins, and terpenoids (Dev et al., 2010).

Based on research by Hengpratom et al. (2020), the fruit extract of O. indicum was known to have anti-adipogenic effects. The fruit extract can reduce the expression levels of PPARγ2, SREBP-1C, FAS, GLUT4, and leptin (Hengpratom et al., 2020). There were also chemical components isolated from the fruit, such as quercetin, apigenin, and baicalein. It was unknown whether the active component played the most crucial role in inhibiting the adipogenesis process in the 3T3-L1 cells. However, these three substances are known to have anti-adipogenic properties (Hengpratom et al., 2020).

In the root bark, the main ingredients are oroxylin, chrysirin, and baicalein (Dev et al., 2010; Harminder et al., 2011). Research conducted by Mangal et al. (2017) revealed that oroxylin A, chrysirin, and baicalein in the bark of roots can inhibit the adipogenesis process in the 3T3-L1 cell line. These three substances were able to inhibit PPARγ and C/EBPα genes significantly. In addition, these three substances were also able to inhibit the expression of other genes such as adipocyte protein 2, a disheveled-binding antagonist of beta-catenin 1, FAS, and SREBP1 in a non-significant way (Mangal et al., 2017).

Given the multitude of chemical contents in the fruit and roots of O. indicum, this could be material for further research in developing substances to treat obesity. The anti-adipogenic effect produced by this plant involves the main pathways of adipocyte differentiation and lipid metabolism; hence, it also has the potential to be utilized for the prevention of obesity.

Spiranthes sinensis

Spiranthes sinensis is a type of endangered orchid found in East and Southeast Asia (Pace et al., 2019). In Indonesia, it can be found in the regions of Sumatra, Java, and the Papua islands. This plant is commonly used as a traditional medicine in East Asia to treat various inflammatory, anti-cancer, anti-diabetes, and other diseases (Fan and Huang, 2019). Research on this plant is relatively rare compared to other medicinal plants in Indonesia. However, many studies discuss S. sinensis. For example, research conducted by Liu et al. (2013) identified phytochemicals in these plants as chalcone, flavonoids, isoflavonoids, rotenoids, 9,10-dihydrophenanthrene, and phenols.

Phenanthrene substances are commonly found in the Orchidaceae family. Research conducted by Shie et al. (2020) was able to isolate six phenanthrene substances in these plants and found that sinensol-C could inhibit the adipogenesis process in the 3T3-L1 cell line. Sinensol-C in S. sinensis can inhibit the adipogenesis process by inhibiting transcription factors, namely PPARγ, C/EBPα, and SREBP-1c. This was indicated by a decrease in gene expression that is specific and influenced by transcription factors such as FAS and FABP4. The mechanism by which this plant extract can inhibit the adipogenesis process is through AMPK activation (Shie et al., 2020). AMPK can inhibit the adipogenesis process by inhibiting the synthesis of SREBP-1c, which then affects the synthesis of PPARγ and C/EBPα (Ahmad et al., 2020; Shie et al., 2020). Based on the above results, research on obesity management could use sinensol-C to involve other orchids in Indonesia.

Adipogenesis is a differentiation of preadipocytes into mature adipocytes which is characterized by abundant lipid droplets and consists of four stages, namely growth inhibition, mitotic clonal expansion, early differentiation, and late differentiation (Moseti et al., 2016) (Fig. 3). Initially, preadipocytes undergo proliferation before entering the growth inhibition phase. Thereafter, induction with phosphodiesterase inhibitor 1-methyl-3-isobutyl xanthine, insulin, and dexamethasone for 3T3-L1 cells is required to make these preadipocytes enter the mitotic clonal expansion stage and, subsequently, differentiate. The initial differentiation stage is controlled by C/EBPβ and C/EBPδ, while C/EBPα and PPARγ regulate the late differentiation stage. Upon completing the differentiation process, mature adipocytes are formed with specialized morphology and function (Ali et al., 2013; Moseti et al., 2016).

Transcription factors play an essential role in the transcription of DNA into RNA, thus having the ability to modulate gene expression (Mota de Sá et al., 2017). Various transcription factors influence the adipogenesis process. Each of these factors has its respective roles in regulating the adipogenesis process, either stimulatory or inhibitory. The example stimulator of adipogenesis includes activator protein-1, KLFs 4 and 5, C/EBPs, SREBP-1, signal transducer and activator of transcription proteins, and PPARγ. Meanwhile, the example inhibitor of adipogenesis includes Wnt signaling-related proteins, GATA transcription factors, KLFs 2, 3, and 7, and preadipocyte factor-1 (Sarjeant and Stephens, 2012). The main adipogenesis transcription factors are PPARγ and C/EBPα. During the adipogenesis process, there is an interaction between the C/EBPs family and the PPAR. C/EBPβ and C/EBPδ induce PPARγ, which regulates adipocyte cell

![Figure 3. Adipogenesis.](image-url)
differentiation programs. C/EBPα and PPARγ induce each other in the form of positive feedback (Ali et al., 2013).

The five plants discussed above inhibit the adipogenesis process in the 3T3-L1 cell line, by inhibiting the main transcription factors, such as PPARγ and C/EBPα (Ali et al., 2013). It has been discussed previously that these two factors are the main effector in the adipogenic process. In addition, several transcription factors which are influenced by the phytochemical content of Indonesian plants are presented in Table 1.

SREBP-1c is an isoform of SREBP-1. It is an important regulator in the adipogenesis process. SREBP-1c plays a crucial role in regulating genes required for lipogenesis and maintaining lipid homeostasis in the body, by increasing the expression of genes such as FAS and LAL. SREBP-1c is also a pro-adipogenic gene that increases the gene expression of PPAR (Ali et al., 2013; Moseti et al., 2016; Mota de Sá et al., 2017).

Meanwhile, FAS and Lipin1 have other functions. FAS plays a pivotal role in de novo lipogenesis and lipid droplet accumulation. It triggers the accumulation of lipids in mature adipocytes, while Lipin1 plays a role in strengthening the bonds between PPARγ and C/EBPα (Borah et al., 2019; Hengpratom et al., 2020; Koh et al., 2008; Moseti et al., 2016).

KLF7 is one of the negative regulators of adipogenesis. KLF7 inhibits clonal expansion during adipogenesis, resulting in decreased lipid accumulation and adiponectin expression in adipocyte cells (Moseti et al., 2016).

It has been previously mentioned that the action of PPARγ and C/EBPα will trigger the process of changing preadipocytes to adipocytes (Ali et al., 2013). This is indicated by the decrease in the expression of various genes due to suppression by medicinal plants. For example, C. roseus inhibits PLN1, FABP4, LPL, ATGL, GLUT4, and adiponectin. L. speciosa inhibits adiponectin. O. indicum inhibits FAS, GLUT4, and leptin, and S. sinensis inhibits FAS and FABP4. All these genes play a very important role in the accumulation of lipids in adipocyte cells. PLN1 plays a role in forming lipid droplets and protecting against lipase; FABP4, ATGL, and LPL are involved in the metabolic process and uptake of lipids, while GLUT4 ensures glucose transport into cells (Borah et al., 2019; Moseti et al., 2016). Meanwhile, adiponectin and leptin play an autocrine role in adipocytes. Adiponectin increases adipogenesis and lipid storage in adipocytes by working to increase the GLUT4 receptor, while leptin regulates intake and energy in the body (Hwang et al., 1997; Stern et al., 2016).

A summary of the anti-adipogenic mechanism of Indonesian medicinal plants is described in Figure 4.

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**Figure 4.** Transcription factors that are affected by Indonesian medicinal plants.
CONCLUSION

Five Indonesian medicinal plants have anti-adipogenic effects by suppressing PPARγ and C/EBPα, as shown in the 3T3-L1 cell line. These medicinal plants are C. roseus, C. odorata, L. speciosa, O. indicum, and S. sinensis. The chemical constituents of these five medicinal plants can become important in obesity management. Further research is needed to identify which active compounds in the plants can be used to treat obesity and to elaborate on the mechanisms by which these compounds inhibit the induction of adipogenic genes.

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AUTHORS’ CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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The authors declare no conflicts of interest.

ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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