Diosmetin and tamarixetin (methylated flavonoids): A review on their chemistry, sources, pharmacology, and anticancer properties

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ABSTRACT
This review begins with an introduction to the basic skeleton and classes of flavonoids. Studies on flavonoids have shown that the presence or absence of their functional moieties is associated with enhanced cytotoxicity toward cancer cells. Functional moieties include the C2–C3 double bond, C3 hydroxyl group, and 4-carbonyl group at ring C and the pattern of hydroxylation at ring B. Subsequently, the current knowledge on the chemistry, sources, pharmacology, and anticancer properties of diosmetin (DMT) and tamarixetin (TMT), two lesser-known methylated flavonoids with similar molecular structures, is updated. DMT is a methylated flavone with three hydroxyl groups, while TMT is a methylated flavanol with four hydroxyl groups. Both DMT and TMT display strong cytotoxic effects on cancer cell lines. Studies on the anticancer effects and molecular mechanisms of DMT included leukemia and breast, liver, prostate, lung, melanoma, colon, and renal cancer cells, while those of TMT have only been reported in leukemia and liver cancer cells. These findings suggest that flavones lacking the C3 hydroxyl group at ring C are more cytotoxic than flavonols having the C3 hydroxyl group. The in vitro and in vivo cytotoxic activities of DMT and TMT against cancer cells involve different molecular targets and signaling pathways. From this study, it is clear that little is known about the pharmacology and anticancer properties of DMT and TMT. The potentials for further research into these aspects of the two lesser-known methylated flavonoids are enormous.

INTRODUCTION
Flavonoids represent the largest family of phenolic secondary metabolites from plants with more than 9,000 compounds reported (Wang et al., 2011). They occur in most herbs, fruits, and vegetables (Kopustinskiene et al., 2020; Panche et al., 2016). These polyphenols have a molecular structure consisting of two benzene rings A and B that are joined by a heterocyclic pyran ring C forming the benzopyrone (C6-C3-C6) moiety (Raffa et al., 2017; Singh et al., 2014). Rings A and C are composed of the chroman (C6-C3) nucleus (Kanadaswami et al., 2005). The basic skeleton along with the functional moieties is shown in Figure 1.

Flavonoids are subdivided into classes including aurones, chalcones, flavonols, flavanones, flavan-3-ols, anthocyanins, and isoflavones (Kar Mahapatra et al., 2015, 2019). The majority of the flavonoids have the B ring linked in position 2 to the C ring (Fig. 1), and they include aurones, chalcones, flavones, flavonols, flavanones, and flavanols (e.g., Guven et al., 2019; Panche et al., 2016; Raffa et al., 2017; Singh et al., 2014). Aurones are a subclass of flavones (Boumendjel, 2003), while chalcones are precursors of flavonoids and isoflavones (Kar Mahapatra et al., 2015). Flavones (e.g., apigenin and luteolin) have a C2–C3 double bond and a 4-carbonyl group but they lack the C3 hydroxyl group at ring C. Flavonols (e.g., fisetin, quercetin, morin, and myricetin) possess all the three functional moieties (Fig. 1). Flavanones (e.g., naringenin, hesperitin, and taxifolin) lack the C2–C3 double bond, while flavanols (e.g., catechin and epicatechin) lack the C2–C3 double bond and the 4-carbonyl group (Guven et al., 2019; Panche et al., 2016). Flavonoids in which the B ring is linked at positions 3 and 4 to the C ring are called isoflavones (e.g., genistein and...
Flavonoids are endowed with health-promoting properties including nutraceutical, pharmaceutical, and cosmeceutical applications (Panche et al., 2016). Pharmacological properties include antioxidant, antimicrobial, antiallergic, anti-inflammatory, anticarcinogenic, and antidiabetic effects (Guven et al., 2019; Raffa et al., 2017). The medical applications of flavonoids involve protection against cancer and other diseases, such as cardiovascular, rheumatic, obesity, high cholesterol, hypertension, and neurological disorders (Ballard and Junior, 2019; Havsteen, 2002). The anticancer effects of flavonoids operate during the stages of initiation, promotion, and progression of carcinogenesis. In the initiation and promotion stages, flavonoids can inhibit cell proliferation (Abotaleb et al., 2019; Ballard and Junior, 2019). At the stage of progression, flavonoids can inhibit proangiogenesis, regulate metastasis, induce cytotoxicity and apoptosis, promote cell cycle arrest, and reverse multidrug resistance (MDR) or a combination of these mechanisms (Abotaleb et al., 2019; Chahar et al., 2011; Raffa et al., 2017). The antitumor activities of flavonoids include the induction of apoptosis, suppression of protein tyrosine kinase activity, antiproliferation, antimetastasis, anti-invasive effects, and antangiogenesis (Kandaswami et al., 2005). Many studies have provided scientific evidence for the anticancer properties of flavonoids in vitro and in vivo (Ren et al., 2013; Wang, 2000). Flavonoids such as quercetin and flavopiridol are now in phase II human clinical trials for different cancers.

When tested against different cancer cell lines, cytotoxicity of various classes of flavonoids based on IC₅₀ values was ranked as flavones > flavonols > flavanones > isoflavones ~ flavanols (Kuntz et al., 1999; Li et al., 2008; Plochmann et al., 2007; Sak, 2014). Flavones have the strongest cytotoxicity over the other groups of flavonoids due to the presence of the C2–C3 double bond, compared to the 4-carbonyl (4-oxo or 4-keto) group and the C3-hydroxy group at ring C (Fig. 1). Disparity exists as stronger cytotoxicity has been reported in flavonols than flavones, for example, quercetin > kaempferol > apigenin (Wang et al., 2018).

The pattern of hydroxylation in ring B influences the degree of cytotoxicity; for example, the ortho-hydroxylated quercetin (3’ and 4’) is three times more cytotoxic than the meta-hydroxylated morin (2’ and 4’). Other factors influencing cytotoxicity are O-methylation and glucuronidation in the A ring which are associated with enhanced cytotoxicity, while a higher number of hydroxyl residues and solubility are inversely correlated with cytotoxicity (Plochmann et al., 2007).

When tested against five different cancer cell lines, flavonoids can be categorized into those with strong and those with weak in vitro cytotoxic effects (Chang et al., 2008). Apigenin, luteolin, and fisetin of the strong category are characterized by having two hydroxyl groups in rings AC, while myricetin and morin of the weak category have three hydroxyl groups in rings AC (Fig. 1). Both naringenin and apigenin share the same molecular structure. Naringenin without the 2,3-double bond displayed weak cytotoxic effects suggesting the importance of the double bond between C2 and C3 (Chang et al., 2008). Genistein and daidzein are isoflavones in which ring B is attached to ring C at C3 instead of C2.

For polymethylated flavonoids (e.g., natsudaidain), a methoxy group at C8 and a hydroxyl group at C3 are essential for their antiproliferative activity of the flavonoids (Kawai et al., 1999). Isoflavones (e.g., genistein and daidzein) are flavonoids in which the B ring is linked in position 3 of the C ring (Chang et al., 2008; Lopez-Lazaro, 2002; Lopez-Lazaro et al., 2002). Generally, isoflavones have weaker cytotoxicity than the other flavonoids linked in position 2. In addition, the sugar moiety of flavonoids (e.g., rutin and isoorientin) reduces their cytotoxic activity (Lopez-Lazaro, 2002; Lopez-Lazaro et al., 2002). In flavonoids, the ring B catechol moiety of flavonoids (e.g., 3’,4’-diOH) and the –OMe group at 5’ are beneficial toward their cytotoxicity, while glycosylation at C5 of ring A has adverse effects on cytotoxicity.
CHEMISTRY AND SOURCES

**Diosmetin**

DMT (4′-methylflavone, luteolin 4′-methyl ether or 5,7,3′-tri-hydroxy-4′-methoxy flavone) is a natural methylated flavone. Its molecular formula is C_{16}H_{12}O_{5} and its molecular weight is 300 g/mol (Patel et al., 2013). DMT has three hydroxyl groups at 5, 7, and 3′ positions (Fig. 2). Being a flavone, the molecule has a C2–C3 double bond and a 4-carbonyl group but lacks the C3 hydroxyl group at ring C. The DMT molecule is structurally similar to that of luteolin with the exception of the 4′-methoxy group in DMT and the 4′-hydroxy group in luteolin. DMT is an aglycone of diosmin or DMT 7-O-rutinoside (Chen et al., 2019a).

DMT has been isolated from many plant species. They include the aerial parts of Soroseres hookeriana (Hooker’s Soroseres) (Meng et al., 2000) and Petroselinum crispum (parsley) (Yoshikawa et al., 2000), Citrus fruit juices (Abad-Garcia et al., 2014), and flowers of Chrysanthemum morifolium (chrysanthemum) (Lin and Harly, 2010; Xie et al., 2009). From the flowers and leaves of Origanum vulgare (oregano), the contents of DMT have been reported to be 0.18 and 0.04 DW: mg/g dry weight (Radošiūnienė et al., 2008). From the ethyl acetate fraction of the methanol extract of Eolecharis dulcis (water chestnut) peel, the content of DMT (30 mg/g) ranked second to that of fisetin (32 mg/g) (Zhan et al., 2016). Glycosides of DMT are commonly found in Citrus fruit juices, notably those of Citrus medica and Citrus bergamia (Caristi et al., 2006; Hostetler et al., 2017).

**Tamarixetin**

TMT (4′-O-methylquercetin, quercetin 4′-methyl ether or 3,5,7,3′-tetrahydroxy-4′-methoxy flavonol) is a natural methylated flavonol with a molecular formula of C_{16}H_{12}O_{8}, and molecular weight of 316 g/mol. TMT has four hydroxyl groups at 3, 5, 7, and 3′ positions (Fig. 2). Being a flavonol, the molecule has a C2–C3 double bond, a 4-carbonyl group, and a C3 hydroxyl group at ring C. It is structurally similar to isorhamnetin (3′-O-methylquercetin) and quercetin. TMT has been isolated from the leaves of Tamarix ramosissima (salt cedar) (Sultanova et al., 2001), Azadirachta indica (neem) (Yadav et al., 2017), and Psidium guajava (guava) (Shao et al., 2014).

PHARMACOLOGY

**Diosmetin**

The anti-inflammatory, antioxidant, and hepatoprotective effects of DMT have been reported (Yang et al., 2017). Other pharmacological properties of DMT include antimicrobial

| Cancer cell line and type | Anticancer effect and molecular mechanism of diosmetin (DMT) |
|---------------------------|-------------------------------------------------------------|
| MDA-MB-468 breast        | Inhibits cell proliferation, causes G1 cell cycle arrest, and exerts cytostatic effects via CYP1 enzyme-mediated conversion to luteolin (Androutsopoulos et al., 2009a) |
| MCF-7 breast              | Inhibits cell proliferation and its cytotoxic effects are dependent on CYP1 enzyme conversion to luteolin (Androutsopoulos et al., 2009b) |
| MDA-MB-231 breast         | Exerts antiproliferative and proapoptotic activities via cell cycle arrest and the mitochondria-mediated intrinsic apoptotic pathway (Wang et al., 2019) |
| HepG2 liver               | Exerts synergistic cytostatic effects and arrest G2/M cell cycle when applied with luteolin via CYP1A-catalyzed metabolism, activation of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK), and P53/P21 upregulation (Androutsopoulos and Spandios, 2013) |
| HepG2 liver               | Induces cell apoptosis by upregulating p53 via the transforming growth factorβ (TGF-β) signal pathway (Liu et al., 2016a) |
| SK-HEP-1 liver            | Inhibits cell metastasis by downregulating the expression levels of MMP-2 and MMP-9 via the protein kinase (PKC)/mitogen-activated protein kinase (MAPK)/metalloproteinase (MMP) pathways (Liu et al., 2016b) |
| HepG2 liver               | Inhibits cell proliferation and induces apoptosis by regulating autophagy via the mammalian target of rapamycin (mTOR) pathway (Liu et al., 2016c) |
| HepG2 liver               | Triggers apoptosis by activation and inactivation of the p53/Bcl-2 pathway and the Notch1/nuclear factor-kappa B (NF-kB) pathway, respectively (Qiao et al., 2016) |
| HepG2 liver               | Inhibits cell proliferation and promotes cell apoptosis and cell cycle arrest by targeting chk2 (Ma and Zhang, 2020) |
| PC-3 and LNCaP prostate   | Suppresses cell proliferation via induction of apoptosis and cell cycle arrest (Oak et al., 2018) |
| NSCLC lung                | Induces apoptosis by producing reactive oxygen species (ROS) and reducing Nrf2 stability via suppression of the PI3K/Akt/glycogen synthase kinase 3 beta (GSK-3β) pathway (Chen et al., 2019b) |
| B16F10 melanoma           | Suppresses tumor progression and metastasis by inducing cell death and inhibiting angiogenesis (Choi et al., 2019) |
| HCT-116 colon             | Suppresses tumor progression and metastasis by inducing cell death and inhibiting angiogenesis (Choi et al., 2019) |
| HCT-116 colon xenograft   | Reduces tumor growth in nude mice by downregulation of Bcl-2 and upregulation of Bax (Koosha et al., 2019b) |
| ACHN renal                | Inactivates and cytotoxicity by reducing protein kinase B (AKT) phosphorylation via p53 upregulation (Qiao et al., 2020) |
| K562 leukemia             | Induces apoptosis via activation of caspases 8 and 3/7 and the death-inducing cytokine tumor necrosis factor alpha (TNFa) (Roma et al., 2019) |

Bax = Bcl-2 associated X protein; Bcl-2 = B-cell lymphoma 2; chk2 = checkpoint kinase 2; CYP = cytochrome P450; JNK = c-Jun N-terminal kinase; ERK = extracellular signal-regulated kinase; GSK-3β = glycogen synthase kinase 3 beta; MAPK = mitogen-activated protein kinase; MMP = metalloproteinase; mTOR = mammalian target of rapamycin; NF-kB = nuclear factor-kappa B; Nrf2 = nuclear factor erythroid 2-related factor 2; PI3K = phosphatidylinoside 3-kinase; PKC = protein kinase C; ROS = reactive oxygen species; TGF = transforming growth factorβ; and TNFa = tumor necrosis factor alpha.
et al., 2000), oestrogenic (Yoshikawa et al., 2000), neuroprotective (Bhatt and Benzeroual, 2013), drug-drug interaction (Bajraktari and Weiss, 2020), osteoblastic (Hsu and Kuo, 2008), and MDR protein inhibitory (van Zanden et al., 2005) activities.

**Tamarixetin**

TMT displays anti-inflammatory (Lesjak et al., 2018; Park et al., 2018), cardioprotective (Fan et al., 2019; Hayamizu et al., 2018), gastroprotective (Yadav et al., 2017), and MDR protein inhibitory (van Zanden et al., 2005) activities.

**ANTICANCER PROPERTIES**

**Diosmetin**

Against Caco-2 and HT-29 and colon cancer cells, the EC\textsubscript{50} values of DMT were 108 and 204 μM, respectively (Kuntz et al., 1999). The cytotoxicity of DMT was 1.2 and 1.8 times weaker than those of luteolin. Against COLO 205 colon cancer cells, the IC\textsubscript{50} value of DMT (82.9 μM) was slightly stronger than luteolin (96.9 μM) while diosmin (>200 μM) did not show any activity (Xie et al., 2009). Against A549 lung cancer cells, the IC\textsubscript{50} value of DMT (101 μg/mL) was weaker than luteolin (59.6 μg/mL) and fisetin (86.5 μg/mL) (Zhan et al., 2016). Besides the structural features of flavonoids, for example, flavones versus flavonols, these results show that cytotoxicity also depends on the type of cancer cells tested. Cytotoxic effects of DMT toward HCT-116 colon cancer cells (3.6 μg/mL) were 14 times more potent than toward CCD-841 normal colon cells (52 μg/mL) (Koosha et al., 2019a).

The anticancer effects and molecular mechanisms of DMT toward different cancer cell lines are listed in Table 1. Against MCF-7 and MDA-MB-468 breast cancer cells, DMT inhibits cell proliferation, arrests G1 cell cycle, and exerts enhanced cytotoxic or cytostatic effects via CYP1 enzyme-mediated conversion to luteolin (Androutsopolous et al., 2009a, 2009b). DMT displays antiproliferative and proapoptotic activities against MDA-MB-231 breast cancer cells via cell cycle arrest and the mitochondria-mediated intrinsic apoptotic pathway (Wang et al., 2019).

When used in combination against HepG2 liver cancer cells, DMT and luteolin exhibit cytostatic effects and arrest G2/M cell cycle via CYP1A1-catalyzed metabolism, P53/P21 upregulation, and JNK and ERK activation (Androutsopolous and Spandidos, 2013). When tested with HepG2 and SK-HEP-1 liver cancer cells, DMT induces cell apoptosis by upregulating p53 via the TGF-β signal pathway (Liu et al., 2016a); inhibits cell metastasis by downregulating the expression levels of MMP-2 and MMP-9 via the PKC/MAPK/MMP pathways (Liu et al., 2016b); inhibits cell proliferation by inducing apoptosis and by regulating autophagy via the mTOR pathway (Liu et al., 2016c); triggers apoptosis by activation of the p53/Bcl-2 pathway and inactivation of the Notch3/NF-xB pathway (Qiao et al., 2016); suppresses cell proliferation; and enhances cell apoptosis and cell cycle arrest by targeting chk2 (Ma and Zhang, 2020).

The anticancer properties of DMT were studied using other cancer cells such as PC-3 and LNCaP prostate [1], NSCLC lung [2], B16F10 melanoma [3], HCT-116 colon [4], ACHN renal [5], and K562 leukemia [6] cell lines (Table 1). DMT suppresses cell proliferation of [1] via induction of apoptosis and cell cycle arrest (Oak et al., 2018); induces apoptosis of [2] by producing ROS and reducing Nrf2 stability via suppression of the PI3K/Akt/GSK-3β pathway (Chen et al., 2019b); and suppresses tumor progression and metastasis of [3] by inducing cell death and inhibiting angiogenesis (Choi et al., 2019). DMT promotes apoptosis, inhibits cell proliferation, and arrests G2/M cell cycle of [4] mediated by the membrane death receptor (Koosha et al., 2019a); reduces tumor growth of [4] in nude mice via downregulation of Bel-2 and overexpression of Bax (Koosha et al., 2019b); promotes apoptosis and cytotoxicity of [5] by reducing AKT phosphorylation via p53 upregulation (Qiu et al., 2020); and induces apoptosis of [6] via activation of caspases 8 and 3/7 and the death-inducing cytokine TNFα (Roma et al., 2018).

**Tamarixetin**

Against A549 and HCC44 lung cancer cells, cytotoxicity of TMT was 19.6 and 20.3 μM, respectively (Sak et al., 2018). Its cytotoxicity was 3.7 and 5.3 times stronger than that of quercetin. The IC\textsubscript{50} values of TMT were comparable to those of isorhamnetin (3’-O-methyl quercetin) with values of 26.6 and 15.9 μM, respectively. Cytotoxicity of TMT against four different leukemia cell lines, Based on IC50 values, cytotoxicity of TMT against four different leukemia cell lines were 5.5 μM for U937 cells, 7.5 μM for HL-60 cells, 7.5 μM for Molt-3 cells and 24 μM for K562 cells (Nicolini et al., 2014). For Molt-3 and HL-60 leukemia cells, IC\textsubscript{50} values were both 7.5 μM. In a study on the antiproliferative effects of quercetin and catechin metabolites in IC\textsubscript{50} values, the cytotoxicity of TMT (82 μM) was comparable to quercetin (85 μM) when tested against Caco-2 colon cancer cells (Delgado et al., 2014). Against MCF-7 breast and BxPC-3 pancreatic cancer cells, cytotoxicity of TMT was 1.5 and 3.0 times weaker than quercetin, respectively. When tested against AGS gastric, B16F10 melanoma, C6 glioma, and HeLa cervical cancer cells using quercetin, 7-O-methylated quercetin, and 3-O-methylated quercetin, TMT exhibited the strongest cytotoxic activity (Darsandhari et al., 2020).

There are only two studies on the anticancer effects and molecular mechanisms of TMT (Table 2). Against doxorubicin-resistant K562/ADR leukemia cells, TMT inhibits cell proliferation, arrests G2/M cell cycle, and induces apoptosis (Nicolini et al., 2014). In another study, the cytotoxicity of TMT toward HepG2 and PLC/PRF/5 liver cancer cells and nude mice tumor xenograft was reported (Xu et al., 2019). In liver cancer cells, TMT suppresses cell viability via apoptosis, lactate dehydrogenase (LDH) release, caspase-3 activation, ROS accumulation, and decreased mitochondrial membrane potential. In liver tumor xenograft, TMT enhances the expression levels of proapoptotic proteins, including Bax and cleaved caspase-3, and inhibits the expression levels of antiapoptotic proteins. Both in vitro and in vivo studies showed that TMT significantly suppressed the phosphorylation of ERK and AKT in liver cancer cells and tumors (Xu et al., 2019).

**Structure–activity relationship (SAR) studies**

There are very few structure–activity relationship (SAR) studies on DMT and TMT related to anticancer activities. In a study of the inhibitory effects of MDR proteins 1 (MRP 1), an important mechanism in MDR during cancer treatment, methylated flavonoids are among the best inhibitors with IC\textsubscript{50} values ranging from 2.7 to 14.3 μM (van Zanden et al., 2005). Inhibition at 25
μM and IC₅₀ values was 84% and 2.7 μM for DMT and 68% and 7.4 μM for TMT. DMT was the strongest, while TMT ranked third. Values of DMT and TMT were stronger than luteolin and quercetin, suggesting that the 4'-methyl ether moieties of DMT and TMT contribute to their inhibitory effects. In another study on the inhibitory effects of flavonoids on NF-κB signaling in MDA-MB-231 breast cancer cells, DMT (3.7%) displayed stronger inhibition than TMT (2.4%) (Amrutha et al., 2014). Inhibitory values of DMT and TMT were stronger than luteolin (3.0%) and much weaker than quercetin (3.7%), respectively.

**CONCLUSION**

Flavonoids are the largest family of phenolic secondary metabolites from plants. They have a molecular structure consisting of two benzene rings (A and B) joined by a pyran ring (C) forming a benzo-pyrene (C₆-C₃-C₆) moiety. The majority of the flavonoids have the B ring linked in position 2 to the C ring, and they can be further divided into classes such as flavones, flavonols, flavanones, and flavanols. Studies have shown that the presence or absence of some functional moieties is associated with enhanced cytotoxicity toward cancer cells. They include C₂–C₃ double bond, a 4-carbonyl group, and a C₃ hydroxy group at ring C and the pattern of hydroxylation (ortho or meta) at ring B.

DMT and TMT are methylated flavonoids. DMT is a methoxyflavone having three hydroxyl groups, while TMT is a methoxyflavonol with four hydroxyl groups. This review on the anticancer properties of DMT and TMT supported the view that methoxyflavonol with four hydroxyl groups, while TMT is a methoxyflavone having three hydroxyl groups. This review on the anticancer properties of DMT and TMT supported the view that flavones without the C₃ hydroxyl group are stronger in cytotoxicity against cancer cells than flavonols with the C₃ hydroxyl group. However, further investigations are needed to confirm the role of the C₃ hydroxy group in cytotoxicity toward cancer cells.

Further clinical research on DMT and TMT is warranted to evaluate their safety and chemopreventive efficacy when used alone or in combination with other chemotherapy agents. Current knowledge of their pharmacokinetics, bioavailability, and SAR studies is meager. Further research on the structural modifications of DMT and TMT is needed for the synthesis of novel derivatives with enhanced inhibitory effects against different cancer cells and reduced cytotoxicity toward normal cells. For lesser-known bioactive compounds, such as DMT and TMT, their use in purified and standardized extracts containing chemical constituents that have the desired pharmacological activity may be the most practical approach. While Western medicine employs pure and single compounds, Chinese medicine (CM) has long used different combinations of compounds in the form of medicinal herbs to treat, ameliorate, and relieve the symptoms of different diseases. CM may have fewer and less severe side effects than single pure drugs, making them especially attractive to consumers. The development and clinical usage of different formulations of DMT and TMT with synergistic anticancer effects, reduced side effects, and acceptable quality control remain a major challenge. Little is known about the pharmacology and anticancer properties of DMT and TMT. The potentials for further research into these aspects of the two lesser-known methylated flavonoids are enormous. This will generate much research interest among medicinal chemists and researchers who are keen on lesser-known flavonoids.

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**AUTHOR 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.

**CONFLICTS OF INTEREST**

The authors report no financial or any other conflicts of interest in this work.

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