ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Effects of 3,3'-Diindolylmethane on Rat Kidney Tissue

3,3'-Diindolylmethane'ın Sıçan Böbrek Dokusu Üzerindeki Etkileri

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ABSTRACT

Aim: *3,3'-diindolylmethane (DIM) is an important digestive product of indole-3 carbinol (I3C) obtained from the Brassica family (broccoli, cauliflower, cabbage, etc.) of vegetables. 3,3'-diindolylmethane is a substrate with potent immune modulatory activity and antitumor, antiviral, and anti-angiogenic effects. This study aimed to evaluate the effects of DIM on rat kidney tissue using histopathologic methods.*

Material and Method: *In the study, 36 males, 16-week-old, and 220–260 gr Wistar albino adult rats were used. Rats were divided into four equal groups: The control group received only corn oil by oral gavage. The other experimental groups received three different doses of DIM dissolved in corn oil, 10 mg/kg DIM (DIM 10 group), 50 mg/kg DIM (DIM 50 group), and 100 mg/kg DIM (DIM-100 group, were administered via oral gavage. Oral gavages were applied to experimental groups for 53 days. At the end of the experiment, all rats were euthanized, and the kidney tissues were dissected. For histopathological examination, the kidney tissue samples were stained with hematoxylin-eosin and Masson–trichrome.*

Results: *Our investigation revealed that the use of DIM at different doses for 53 days caused dose-dependent histopathological changes, including apoptotic to necrotic changes, interstitial inflammation to fibrotic connective tissue changes, and cast formations starting from the Henle loops and spreading to the renal tubules.*

Conclusion: *These histopathological changes could have occurred due to a DIM-mediated increase in reactive oxygen species (ROS). Further biochemical, molecular, and ultrastructural studies are needed to clarify these findings.*

Key words: *3,3'-diindolylmethane; indole-3 carbinol; kidney; reactive oxygen species (ROS)*

ÖZET

Amaç: *3,3'-diindolilmetan (DIM), Brassica ailesi (brokoli, karnabahar, lahana vb.) sebzelerinden elde edilen indol-3-karbinolün (I3C) önemli bir sindirim ürünüdür. DIM, güçlü bağışıklık modülatör aktiviteye sahip bir substrat olup antitümör, antiviral ve anti-anjiyogenik etkilere sahiptir. Bu çalışmada, DIM'in sıçan böbrek dokusu üzerindeki etkilerinin histopatolojik yöntemlerle değerlendirilmesi amaçlanmıştır.*

Materyal ve Metot: *Çalışmada 16 haftalık, 220–260 gr ağırlığında toplam 36 erkek Wistar albino erişkin sıçan kullanılmıştır. Sıçanlar dört eşit gruba ayrılmıştır: Kontrol grubuna sadece oral yolla mısır yağı verilmiştir. Diğer deney gruplarına mısır yağı içinde çözünmüş üç farklı dozda DIM uygulanmıştır: 10 mg/kg DIM (DIM 10 grubu), 50 mg/kg DIM (DIM 50 grubu) ve 100 mg/kg DIM (DIM 100 grubu). Oral uygulamalar deney gruplarına 53 gün boyunca uygulanmıştır. Deney sonunda tüm sıçanlar ötanazi edilmiş ve böbrek dokuları çıkarılmıştır. Histopatolojik inceleme için böbrek doku örnekleri hematoksilen-eozin ve Masson-trikrom ile boyanmıştır.*

Bulgular: *Çalışmamız, 53 gün boyunca farklı dozlarda DIM kullanımının, doz bağımlı histopatolojik değişikliklere neden olduğunu ortaya koymuştur. Bu değişiklikler, apoptotik ve nekrotik değişimlerden, interstisyel enflamasyondan fibrotik bağ dokusu değişimlerine ve Henle kıvrımlarından başlayarak renal tübüllere yayılan kast oluşumlarını içermektedir.*

Sonuç: *Bu histopatolojik değişiklikler, DIM'in reaktif oksijen türlerini (ROS) artırması sonucu ortaya çıkmış olabilir. Bu bulguları aydınlatmak için ileri düzey biyokimyasal, moleküler ve ultrastrüktürel çalışmalara ihtiyaç duyulmaktadır.*

Anahtar kelimeler: *3,3'-diindolilmetan; böbrek; reaktif oksijen türleri (ROS)*

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Introduction

Vegetables from the Cruciferous (Brassicaceae) family contain considerable amounts of a bioactive phytochemical agent with inhibitory and therapeutic potential for tumorigenesis[1]. Broccoli, cabbage, and other cruciferous family members contain the bioactive compound indole-3-carbinol (I3C). I3C is chemically converted from the bioactive compound to the main condensation product, 3.3'-diindolylmethane (DIM), in the aqueous and acidic in vivo gastric environment[2].

3,3'-diindolylmethane is an agent that has antiviral, anti-angiogenic, and antitumor effects[3–6]. Its different pleiotropic effects on cancer cells are shown in many studies[3,7,8]. 3,3'-diindolylmethane functions by inhibiting survival signals on cells and activating multiple death pathways simultaneously. It has been reported that women who consume high levels of cruciferous vegetables have a reduced risk of cervical, endometrial, and breast cancers[5,9,10]. 3,3'-diindolylmethane causes the disruption of cell proliferation in prostate, colon, breast, cervical, and pancreatic cancers that is mediated by stimulating multiple signaling pathways. These signaling pathways inhibit tumor cell migration, invasion, and metastasis and trigger apoptotic cell death[11–14]. 3,3'-diindolylmethane promotes apoptosis by stimulating caspase-3 activity and changing the ratio of Bcl-2/Bax expression[8]. On the other hand, DIM provides a strong immunomodulatory effect by triggering the interferon-gamma signaling pathway, which promotes interferon-gamma and cytokine production[2,6,11,15]. With this effect, DIM plays a chemotherapeutic role in viral diseases such as rotavirus-induced gastroenteritis, respiratory syncytial virus, and HPV infection[16]. Rouse et al., in their experimental study, demonstrated the ameliorative effect of DIM against autoimmune encephalomyelitis, with apoptosis caused by the activation of T cells[17]. Some studies have shown that DIM can attenuate acute liver failure by regulating microRNAs to target IRAK4 and suppress Toll-like receptor signaling, highlighting its potential to protect against liver damage[18]. Furthermore, DIM has been explored for its neuroprotective properties, promoting the formation of brain-derived neurotrophic factor (BDNF) and antioxidant enzymes via the TrkB/Akt pathway activation, which can protect against oxidative stress-induced apoptosis in neuronal cells[19]. This suggests that DIM may have applications in neuroprotection and potentially in neurodegenerative diseases.

On the other hand, there are concerns regarding the use of DIM. Some studies have suggested a possible association between DIM, pulmonary embolism, and deep venous thrombosis[20]. Moreover, studies have linked DIM to potential negative effects on estrogen and androgen physiology, while its effects on cancer risk remain unclear[21]. While DIM shows promise in various health aspects, it is crucial to consider potential negative effects, indicating the need for further investigation.

In various contexts, 3.3'-diindolylmethane (DIM) has been linked to increased reactive oxygen species (ROS). 3,3'-diindolylmethane has been shown to increase the release of ROS from mitochondria, which causes p21 to be increased in human breast cancer cells[22]. Additionally, murine peritoneal macrophage cultures have linked DIM to promoting ROS production, indicating its potential to stimulate immune function through ROS modulation[6]. Furthermore, by affecting lipid ROS levels, DIM induces ferroptosis in gastric cancer cells, suggesting a role in ROS-mediated cell death mechanisms[23]. Our research aimed to examine the impact of long-term use of diindolylmethane (DIM) on the cellular structure of rat kidney tissues.

Material and Method

Experimental Protocol

The study was approved by the Committee for Institutional Animal Care and Use of Bingöl University Local Board of Ethics (Decision no: 125915/2023).

Thirty-six male, 16 weeks old, and weighing 220–260g Wistar albino rats were used in the study. The animals were obtained from Bingöl University Medical Experimental Research and Application Centre (Bingöl, Türkiye). The rats were housed at 40–60% humidity, at 24°C, with a 12 h light-dark cycle, and at standard laboratory conditions with available pellet chow (Bayramoğlu Food Co., Erzurum, Türkiye) and ad libitum water. Rats were divided into four equal groups. The control group received only corn oil (Oruçoğlu Co., Afyonkarahisar, Türkiye), which was given by oral gavage. The DIM 10 group received 10 mg/kg DIM (Sigma-Aldrich Co., St. Louis, MO, USA), DIM 50 received 50 mg/kg DIM, and DIM 100 received 100 mg/kg DIM that dissolved in corn oil by oral gavage[16].

Experimental groups received DIM for 53 days. At the end of the experiment, all rats were euthanized with sevoflurane inhalation anesthesia, and the kidney tissues were removed for histopathologic examinations.

Histopathological Analysis

Tissue Processing

We first fix the kidney tissue samples in an immersion solution (10% formalin) for 72 hours to stabilize cellular proteins and structures. Later, we dehydrated the kidney tissues with increasing alcohol concentrations to ensure dehydration and prevent shrinkage. We then passed the samples through the Xylol series for clearing and transparency. Finally, we passed the samples through the paraffin series and embedded them in paraffin wax (Agar, Cambridge, UK). The embedded tissue blocks were sectioned at 4 µm thickness using a microtome (Leica RM2125RT).

Hematoxylin-eosin Staining

We deparaffinized the tissue sections in a series of xylene, then rehydrated them using descending alcohol concentrations. We immersed the sections in Mayer's hematoxylin solution for five minutes. We rinsed the tissue sections and immersed them in a weak acid-alcohol solution for one to two seconds to remove excess dye. Next, we immersed the tissue sections in the eosin Y solution for one minute. After staining with eosin and rinsing, we dehydrated the sections by gradually increasing the alcohol concentration to eliminate excess water. Later, we cleared the tissue sections in xylene to make them transparent and suitable for mounting. We mounted the dehydrated and cleared sections onto slides using entellan, a mounting medium. We placed a coverslip over the sections. We then examined the tissue sections under a light microscope (Nikon Eclipse i50, Tokyo, Japan).

Masson-trichome Staining

Like H&E staining, paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated through descending alcohol concentrations. Sections were first stained with Weigert's iron hematoxylin solution for eight minutes. After rinsing, we differentiated excess hematoxylin dye in an acid-alcohol solution for one to two seconds. We rinsed the tissue sections and stained them with Biebrich Scarlet-Acid Fuchsin solution. Afterward, we treated the sections with a phosphomolybdic acid solution for five minutes to eliminate the excess acid dye from the tissue. Finally, the sections were stained with an aniline blue solution. We dehydrated the tissue sections using an increasing alcohol concentration series, cleared them in xylene, mounted them onto glass slides, and cover-slipped them using a mounting medium. We then examined the tissue sections under a light microscope (Nikon Eclipse i50, Tokyo, Japan).

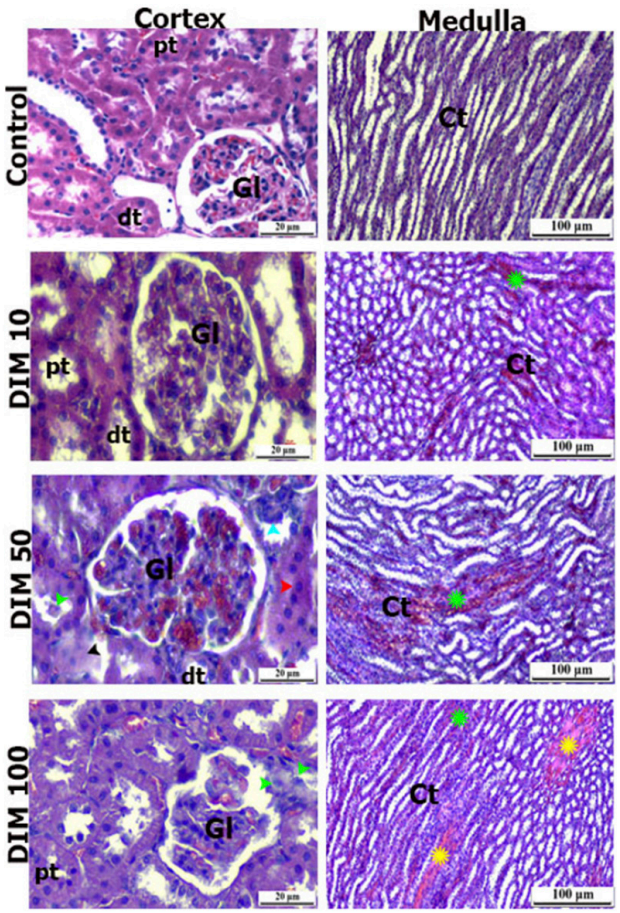

Figure 1. *Hematoxylin and eosin staining of kidney tissues of all groups,* ***Gl:*** *glomerulus,* ***dt:*** *distal tubule,* ***pt:*** *proximal tubule,* ***ct:*** *collecting tubules,* ***green star:*** *congestion in collecting tubules,* ***yellow star:*** *cast formation in Henle loops,* ***black arrowhead:*** *cellular swelling (finding of necrosis),* ***green arrowhead:*** *nuclear fragmentation (finding of apoptosis),* ***blue arrowhead:*** *a group of tubuler cells is experiencing cell and nuclear shrinkage (finding of apoptosis),* ***red arrowhead:*** *intense eosinophilic staining (finding of apoptosis).*

Results

Histopathological Results

In the histopathologic examinations, the kidney tissues of the control group had normal histological structures (Figures 1 and 2). 3,3'-diindolylmethane 10 group showed some destruction of the normal architecture of kidneys. There was mild glomerular and

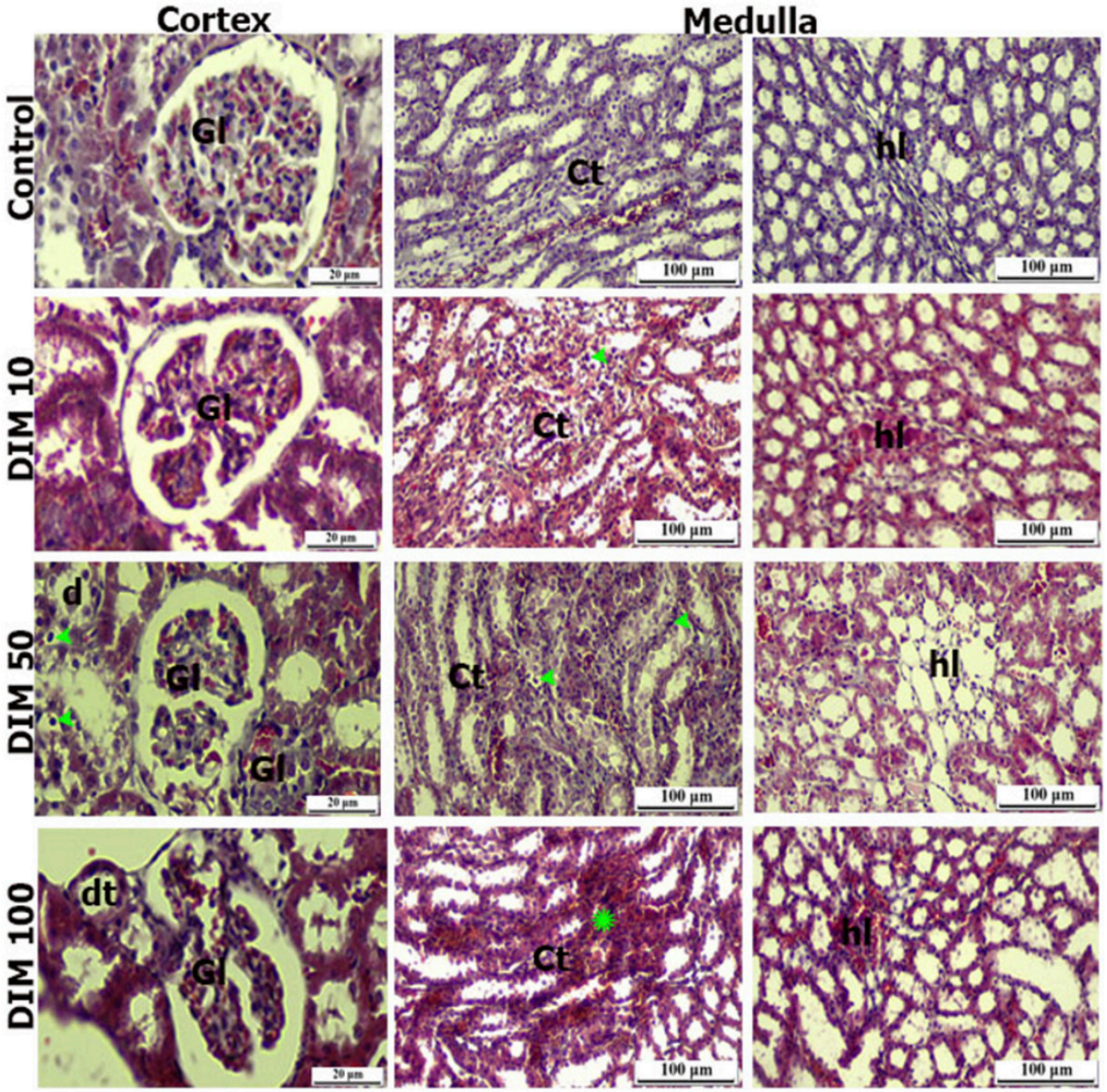

Figure 2. *Masson's trichrome staining of kidney tissues of all groups,* ***Gl:*** *glomerulus,* ***dt:*** *distal tubule,* ***ct:*** *collecting tubules,* ***hl:*** *Henle loop,* ***green arrowhead:*** *inflammatory cell,* ***green star:*** *necrotic and fibrotic change.*

tubular damage, interstitial inflammation, cast formation of Henle loops, and congestion in rat kidneys. Besides this, apoptotic changes in renal tubular cells and glomeruli were remarkable (Figures 1 and 2). In kidney tissues of the DIM 50 group, there was moderate glomerular and tubular damage, interstitial inflammation, cast formation of Henle loops, and congestion. Different from the DIM 10 group, there was swelling in renal tubular cells and dilatations in Henle loops (Figures 1 and 2). Kidney tissues of the DIM 100 group had a large amount of destruction of the normal architecture of kidneys. This group had extensive glomerular and tubular damage, interstitial inflammation, cast formation of Henle loops, and congestion. In addition to DIM 10 and 50 group findings, cast formation was seen in renal tubulars. Moreover, interstitial fibrous tissue formation and necrosis were significant in this group (Figures 1 and 2).

Discussion

3.3'-Diindolylmethane (DIM) is a compound found in cruciferous vegetables, known for its potential health benefits and cancer prevention[24]. It may also help in immune system support, anti-inflammatory properties, and detoxification[24–27]. However, more research is needed to understand its mechanisms of action and potential benefits fully. This study aimed to investigate the effects of DIM on rat kidney tissues at the cellular level.

The findings of studies related to DIM and kidneys argue that DIM has protective and healing effects on the kidney[28–31]. However, our study findings contradict the previous studies. Therefore, it is necessary to clearly explain the reason or reasons for which our findings are based.

Our study findings showed that DIM causes adverse changes in kidney tissue, from dose-dependent apoptosis to necrosis. So, what could be the reason for the study findings we obtained, while some studies contradict our findings and argue that DIM has protective and healing effects on the kidney? What can trigger apoptosis and necrosis at the tissue and cell level? Studies have demonstrated that DIM inhibits the growth and invasion of cancer cells, induces apoptosis, and suppresses inflammatory responses[32]. Roh et al.[33] suggested that DIM induced apoptosis in testicular tissue. Roh et al.[33] found that 3.3′-diindolylmethane causes immunotoxicity in neonatal mice by inducing apoptosis in splenocytes. Goldberg et al. discovered that modified versions of 3.3′-diindolylmethane (DIM) with a ring substitution cause programmed cell death and necrosis in both androgen-dependent and androgen-independent prostate cancer cells[34]. Ye et al. reported that 3.3'-diindolylmethane potentiates tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis of gastric cancer cells. Reactive oxygen species (ROS) can affect cell fate by promoting apoptosis. Simon et al.[35] showed that ROS significantly induces apoptosis under physiological and pathological conditions. Gach et al.[36] suggested that ROS promotes the initiation of apoptosis. Higuchi et al.[37] reported that caspase 3-like protease could induce ROS to cause both apoptosis and necrosis. Furthermore, Morgan et al.[38] suggested that necrotic cell death depended on the ROS. Xue et al.[6,39] showed increased malondialdehyde (MDA) levels mediated by DIM, suggesting that DIM induces ROS to increase MDA subsequently. Tripathi et al.[40] reported that ROS could affect cell fate by triggering T cells for apoptosis. The dose-dependent apoptotic and necrotic changes in our findings may be due to oxidative stress caused by increased DIM-mediated ROS.

Our study showed that DIM causes dose-dependent damage, including changes from interstitial inflammation to fibrotic connective tissue. In contrast to our findings, Xia et al.[29] reported that DIM reduced kidney damage. They detected decreased numbers of vimentin, α-SMA, fibronectin, and collagen I-positive cells, whereas they also reported increased numbers of E-cadherin-positive cells from immunohistochemical examinations. This study suggests that DIM treatment reduced interstitial fibrosis by suppressing local fibroblast activation. Martínez-Klimova et al.[41] reported that DIM prevented epithelial-to-mesenchymal transition in their study. Apart from these two studies, no others in the literature show if DIM causes interstitial inflammation and fibrosis in the kidney tissue.

In our study, a dose-dependent cast formation was observed in the renal tissue of DIM application, starting from the Henle loops and progressing to the renal tubules. Leibelt et al.[39] reported that numerous large hyaline casts were found in the kidney tubules of the DIM treated group, in their study. This single study examined only one negative DIM effect on kidney tissues in the literature. Our study supports the findings of Leibelt et al.[39] but different from Leibelt et al.[39], hyaline cast formations are also seen in the Henle loops and begin first in the Henle loops in low doses and progress to the renal tubules with increasing DIM dose. Furthermore, our study showed many histopathological changes that were negative effects of DIM treatment.

Conclusion

A wealth of research in the literature explores DIM's protective and advantageous effects on different types of tissues. Nevertheless, our study findings indicate that long-term use of DIM significantly increases the induction of histopathological alterations. The histological alterations in rat kidney tissue may be attributed to increased reactive oxygen species (ROS) mediated by DIM. To gain a deeper understanding, additional investigations involving biochemical, molecular, and ultrastructural analyses are required.

Declaration of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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Authors' Contributions

Study concept and design: SNP. Creation of experimental groups and experimental practices: S. N. P, SYY, H. G. Histopathological Analyzes: S. N. P, SYY, H. G, TD. Evaluating the results and writing the manuscript: S. N. P, SYY, H. G, TD.

Ethical Approval

Study approval was obtained from the Committee for Institutional Animal Care and Use of Bingöl University Local Board of Ethics (Decision no: 125915 on 05 October 2023).

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