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Distribution of *Corchorus golden mosaic virus* (CoGMV) in jute (*Corchorus capsularis* L.)

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Abstract
Jute plants were examined using in situ hybridization to determine the distribution pattern of *Corchorus* golden mosaic virus (CoGMV). When CoGMV was injected into the leaf petiole of a plant, it spread and multiplied. It then spread to the plant's whole body. After hybridization with the CoGMV specific probe, signals are found in the spongy mesophyll cells of the leaves and the nuclei of the palisade. Hybridization signals are detected in the flowers of diseased plants, and these signals come from the various floral organs, including the sepals, petals, stamens, and ovary. The examination of CoGMV seed localization, hybridization signals seen from the testa tissues, the endospermic cytoplasm surrounding the embryo sac border wall, cotyledonary tissues, and the embryo. So far as we know, this is the first report to describe where CoGMV is found in both the living parts of jute plants and their reproductive parts.

Keywords: CoGMV, In situ hybridization, *Corchorus capsularis*

1. Introduction
Jute (*Corchorus capsularis* L.) is a versatile, environmentally resilient, and biodegradable lingo-cellulosic fiber producing plant that belongs to the Tiliaceae or, more lately, Malvaceae family. It is mostly grown in India and Bangladesh. These two countries account for about 98 percent of global agricultural production (Das *et al*. 2012; Kundu, 1951) [3, 11]. Bangladesh ranks second in terms of jute production among the world's jute-growing countries (Samanta *et al*., 2011) [18]. Jute exports play a significant role in Bangladesh's economy. Roughly 6% of overall foreign currency revenues come from exports. Bangladesh continues to lead the world in exporting jute and jute products.
Jute is susceptible to a variety of diseases, the most devastating of which is leaf mosaic. Finlow (1917) [6] was the first to report on this disease. The jute leaf mosaic is widespread throughout the world's leading jute-producing nations, particularly Bangladesh, Myanmar, and India. (Ghosh and Som, 1998) [9]. Jute leaf mosaic has long been thought to be one of the most important problems with jute farming. Symptoms of jute leaf mosaic disease include green and chlorotic mixed regions that provide a yellow mosaic appearance. There are many ways that the disease affects the quality and quantity of fiber made from jute. It reduces the plant's chlorophyll content as well as its height (Ghosh *et al*., 2011) [7]. Some *C. capsularis* cultivars have a 50% incidence of the disease, and a survey shows that the infection reduces the plant height by 20%, which has a negative impact on fiber yield (Ghosh *et al*., 2008) [8].
The jute leaf mosaic virus is a member of the genus Begomovirus, which is in turn a member of the *Geminiviridae* family. The biggest category of plant viruses is the *Geminiviridae*, which is defined by twinned icosahedral particles of around 18 nanometers X 30 nanometers in size (Stanley *et al*. 2005) [20] and encapsulating a single-stranded circular DNA (ssDNA) genome. The begomovirus is a genus of *Geminiviridae* categorized according to its insect vector, genomic organization, and the host range (Padidam *et al*., 1995) [16]. Begomoviruses are monopartite or bipartite viruses that are spread by the whitefly *Bemisia tabaci* and infect dicotyledonous plants. Most begomoviruses have a bipartite genome structure with two parts, DNA-A and DNA-B, each 2.5–3 kb long. Both DNA-A and DNA-B share a common region that is nearly 200 nucleotides long, highly conserved, and contains repeating motifs (iterons) that act as sequence-specific Rep-binding sites (Moffat, 1997) [14]. While the DNA-A component of bipartite begomoviruses is crucial in replication and virions generation, nuclear localization, systemic infection, host range determination, and symptom expression are all dependent on the DNA-B component (Lazarowitz, 1992) [12]. They also have a single piece of genomic DNA that is similar to DNA-A and has all the proteins needed for

replication, gene expression, insect transmission, systemic infection, and so on. This is called a monopartite begomovirus, and it is different from other begomoviruses (Stanley *et al*., 2005) [20].

Viruses frequently spread systemically throughout their hosts, infecting cells distant from the site of infection. When a virus infects a certain type of cell or tissue, it is called "tissue tropism" (Tyler and Fields, 1996) [22]. Viruses seem to be transmitted symplastically through pre-existing cytoplasmic connections created by plasmodesmata (Carrington *et al*., 1996) [2]. A plant virus must be able to enter and move through the host's circulatory system in order to spread to other parts of the plant. This is called a vascular tissue tropism. When viruses are only found in the vascular system, they are called "phloem-limited viruses" because of their location. On the other hand, certain plant viruses have the ability to infect cells in the mesophyll tissue and spread outside of the vascular system. Such viruses show a strong preference for mesophyll tissue over other forms of tissue. The variables that affect a plant virus's ability to penetrate the vascular system have received little attention (Nelson and van Bel, 1997) [15]. Even though the bipartite geminiviruses of the begomovirus genus synthesize movement proteins with almost the same amino acid sequences, they show a wide range of tissue tropisms in different tissues. In situ hybridization was used in this study to look at how CoGMV was distributed in jute. In this paper, we present the distribution pattern of CoGMV in shoot apical meristems (SAM), floral organs, and vegetative parts of the jute plant and discuss the implications of the findings.

2. Materials and Methods

2.1 CoGMV inocula preparation

For the purpose of constructing an infectious clone of CoGMV DNA A (Accession no. AB849292), the EcoRV-to-KpnI (approximately 1.7 kb) segment of the DNA A clone (1.0 mer) was separated and removed by gel electrophoresis, and the remainder (0.4 mer) of the clone was purified, digested with T4 polymerase, and self-ligated, which was validated as pMD20A0.4mer. In this case, the pMD20A0.4mer was cut with NheI to make it easier to work with. Then, it was ligated with the full-length DNA A component that had been released by NheI to make the pMD20A1.4mer. Restriction digestion with AflII was used to confirm the orientation of the constructs. The 3.7 kb band (1.4 mer) that came out of the recombinant pMD20 clone of DNA A after it was cut with HindIII and EcoRI was cloned into pRI201-AN-GUS. The resulting clone was called pRI1.4A.

A similar strategy was used to create the CoGMV DNA B (Accession no. AB849292) infectious clone. Gel electrophoresis was used to isolate the BglII-to-KpnI (0.5 kb) fragment from the DNA B clone (1 mer). To make pMD20B0.8mer, the remainder (0.8 mer) of the clone was purified, processed with T4 polymerase, and self-ligated. The pMd20B1.8mer was made by combining the FbaI-cut pMD20B0.8mer with the full-length DNA B component released as FbaI fragment. Restriction digestion with PvuII was used to confirm the orientation of the constructs. The 4.9 kb band (1.8 mer) that came out of the recombinant pMD20 clone of DNA B after it was cut with HindIII and EcoRI was cloned into pRI201-AN-GUS. The final clone was called pRI1.8B.

2.2 Plant Material

During the experiment, CVL-1 jute seedlings were grown in small pots in a growth chamber where the temperature was kept at 22±2 °C and the light/dark cycle was 16/8 hours. Agroinoculation was performed on jute seedlings that were in the third and fourth leaf stages. CoGMV DNA A and cognate DNA B were agroinoculated. It took 48 hours for the *Agrobacterium tumefaciens* strain C58C1 cells with the infectious pRI1.4A and pRI1.8B constructs to grow in Luria Bertani medium (pH 6.8) with rifampicin (100 µg/ml) and kanamycin (50 µg/ml) added. The Agrobacterium cells were collected and resuspended in MES buffer, which contained 10 mM 2-(N-morpholino) ethanesulfonic acid (MES), 10 mM Magnesium chloride (MgCl2) and 100 µM acetosyringone. Using a scalpel edge, equal quantities of DNA A and B were mixed (1.0 OD) and inserted into the leaf petiole. Plants that had received agroinoculation were kept at a temperature of 22±2 °C and shielded from pests. Plants that were displaying symptoms were kept in a room with a regulated environment.

2.3 Hybridization probes

A DIG-11UTP NTP mix and T3 RNA polymerase (both from Roche Diagnostics GmbH, Mannheim, Germany) were used to create the digoxygenin (DIG)-labeled minus-stranded CoGMV Rep riboprobes. The Xba I-linearized plasmid pBluSK14Rep served as the template for the *in vitro* transcription, which was carried out according to the manufacturer's procedure. DIG-labeled plus-strand Rep of CoGMV was generated *in vitro* using the Xba I-linearized plasmid pBluSK15Rep as a template.

2.4 Plant tissue preparation

Processing of jute plant samples was carried out in accordance with the Sainte-Marie method but with certain modifications (Sainte-Marie 1962) [17]. FAA (10% formaldehyde, 50% ethanol, and 5% acetic acid) was used to fix the samples overnight at 4°C. Following the steps of dehydration, infiltration, and xylene replacement, the samples were embedded in paraffin (Paraplast Plus, Oxford Labware). A rotary microtome was used to cut paraffin slices (10–12 µm) that were then put on slide glasses coated with aminopropylytriethoxy silane (APS; Matsunami Glass, Osaka, Japan). After 5–10 minutes at room temperature, the sections flattened, and excess water was brushed away from the edges using an absorbent tissue. When the sections were dry, the slides were kept overnight on a warming plate at 42-44°C.

After dewaxing the sections twice in xylene for 10 minutes each and shaking them gently, they were washed in 100% ethanol (twice, 5 minutes each). A graded alcohol series of 90%, 70%, 50%, and 30% washed the slides, and then sterile water was used to rinse them. The slides were then treated at 37°C for 30 minutes with 2 µg ml-1 proteinase K (100 mM Tris, pH 7.6, 50 mM EDTA). Slides were washed twice in phosphate buffered saline (PBS), then dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, 100%) followed by sterile distilled water. A water aspirator was used to dry the sections in a vacuum for 1 hour.

2.5 In situ hybridization

Prehybridization was performed in a humidified box at 55°C for 1 hour using a solution comprising 50% deionized formamide, 0.3 M NaCl, 10% dextran sulphate, 1x Tris-

EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5), 1 µg $µl^{-1}$ yeast tRNA and 1x Denhardt's solution. A total of 250 µl of a new hybridization mixture containing 300ng mL^{-1} DIG-labeled RNA probe was given to each slide after prehybridization, and the slides were then incubated overnight at 55 °C. For 15 minutes, slides were washed three times in 2x SSC (1x SC; 150 mM NaCl, 15 mM Na citrate, pH 7.0) at 50 °C, it was then incubated with 20 $µgml^{-1}$ RNase A diluted in 15 mM Tris, 0.5 M NaCl, and 5 mM EDTA, pH 7.5. A total of two washes with 0.2x SSC were performed at room temperature on the slides (15 minutes for each change). The sections were treated for one hour at room temperature with Anti-Digoxigenin-AP, Fab fragments (Roche; 1:1000 dilution in DIG buffer; 100 mM Tris, 150 mM NaCl, pH 7.5). We then put the sections in the dark for a while with the color substrate solution (100 µl NBT/BCIP, Roche) after they had been washed with DIG buffer. When there was a lot of color, sections were put together and looked at with a microscope. The observations were confirmed by looking at the sections in order.

3. Results

3.1 CoGMV is not capable of invading shoot apical meristem

In situ hybridization was performed to recognize GoGMV-infected cells in longitudinal slices of shoot apical meristems (SAM) derived from jute plants that had been injected with plasmids harboring the CoGMV A and B elements via *Agrobacterium tumefecience*. CoGMV was not detected in SAM of jute plants that had been agroinoculated. Plants that had been agroinoculated with the virus were found to have the virus in their vascular tissues (Fig. 1), but no hybridization signal was found in plants that had not been infected.

3.2 CoGMV can spread into the plant system and mesophyll of infected plants

CoGMV might be inside the nucleus of phloem tissue because it is synthesizing in the nucleus of phloem tissue and importing CoGMV RNA from other, non-vascular tissue. In order to determine CoGMV's dispersion throughout the plant system, in situ hybridization was used to determine its presence. All of our results strongly showed that CoGMV is transported by the phloem during long-distance migration (Fig. 2).

3.3 CoGMV is present in all floral parts

In this study, the presence of CoGMV in jute flower organs was looked into, and hybridization signals from infected sections of flower organs were found. CoGMV was found in a few sepal cells of growing jute flowers, but was completely missing from all petals, stamens, styles, and ovary cells. The infection of the unfertilized ovule was shown by patches of infected tissue on the ovule's edges, which meant it had been infected. In mature flowers of jute, CoGMV was present in all parts of the flower: sepal, petal, stamen, and ovary (Fig. 3), and CoGMV has the ability to invade the placenta and ovule. The virus spread from the vascular-associated tissue to the surrounding tissue, where it gradually penetrated the tissue.

Furthermore, total DNA recovered from the sepal, petal, androecium, and gynoecium of CoGMV-[BD: Din:10] infected jute was tested using polymerase chain reaction (PCR). Six out of six flowers gave a positive reaction for all the floral parts, which confirmed a similar distribution pattern as in the in-situ hybridization test.

3.4 CoGMV is present in seeds

We used an in-situ hybridization method to look at how CoGMV was spread in seeds by taking serial sections of both diseased and healthy seeds. About 89 seeds from the CoGMV infected jute plant were harvested and used for analysis with the presence or absence of CoGMV in them. It was found that 16 seeds that had been treated with the in-situ hybridization method had different CoGMV distributions in different parts of them. In this picture, the testes, the endospermic cytoplasm that surrounds the embryo sac border wall, cotyledonary tissues, and the embryo are all infected (Fig. 4). The site and physiological activity of the testa and endospermic cytoplasm, as well as its vulnerability to viral infection, all point to the testa and endospermic cytoplasm as a possible pathway for CoGMV to directly infect jute embryos.

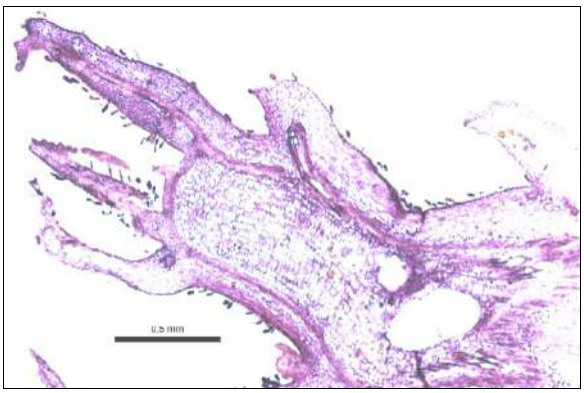
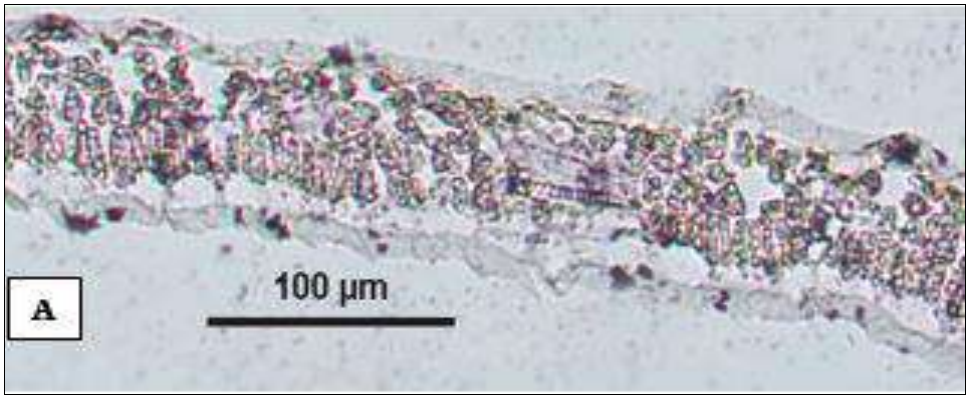


Fig 1: CoGMV distribution in the jute shoot apical meristem (SAM). The bar displays 0.5 mm



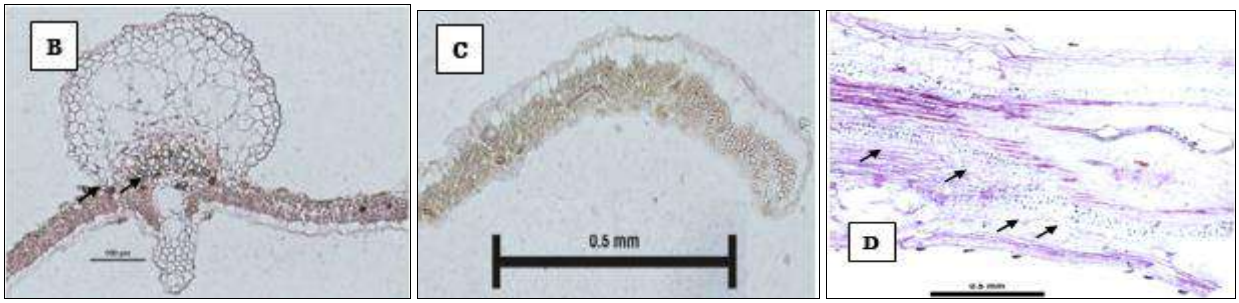


Fig 2: In Situ Localization of CoGMV virus. A and B: Cross-sections of CoGMV-infected leaves after hybridization with a CoGMV-specific probe. Signals were seen in the nuclei of palisade and spongy mesophyll cells. C. A leaf section from a healthy jute plant. D. A stem section from an infected plant stem with the CoGMV virus. The bar indicates the size of the figure.

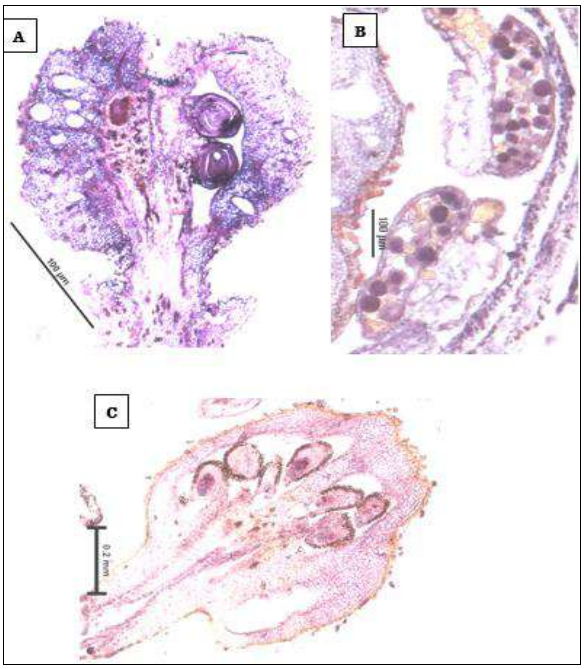


Fig 3: The CoGMV is spread out in jute flowers at the opening stage. A. Cross-section through an infected ovary of a jute plant caused by the CoGMV. B. A highly magnified cross-section of an infected jute flower caused by the CoGMV. C. The ovules and ovary wall of a normal jute plant are shown in this longitudinal segment of the ovary. The size of the figure is shown by the bar

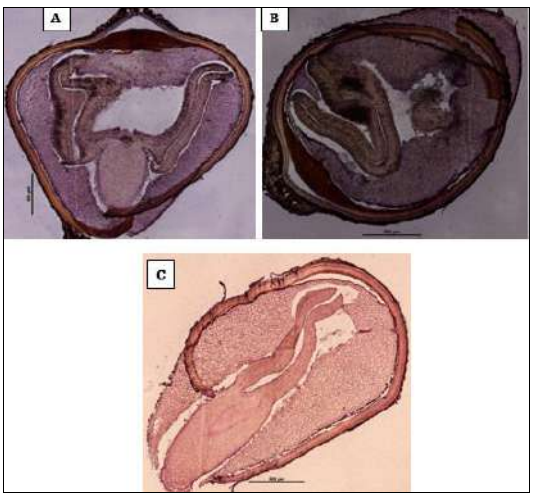


Fig 4: CoGMV prevalence in jute seeds. A-B. Section of CoGMV infected jute seeds. C. A section of healthy jute seed. The bar indicates the size of the figure

4. Discussion

In situ hybridization was utilized to demonstrate that begomovirus CoGMV and jute have a lot of features that make it a good way to figure out how the virus spreads in jute plants and seeds. Viral infections are linked to the host cell machinery at all times. The interaction between host and viral components is what causes all viral phenotypes to emerge. Our findings are consistent with those of Wang *et al*. (1996) [24] and Ding *et al*. (1999) [4], who found that the tissue tropism of the *bean dwarf mosaic virus* depends on the growing period of the host and that environmental variables like temperature may alter the tissue tropism of plant viruses.

Our in-situ hybridization investigation of infected jute plants established unequivocally that CoGMV is incapable of invading SAM, although CoGMV was discovered in the leaf primordium (Fig. 1). Sunter and Bisaro (1992) [21] did an early study, and they found that TGMV didn't seem to be in SAM of plants that had been infected. Those plants that had the apical meristem with the newest pairs of leaf primordials didn't have any problems with cell wall structures. In shoot tips of plants that had been infected with TGMV, no TGMV was found by bioassay, and TGMV-free plants were grown from the shoot tips that had been cut off. The plant that developed from the bigger shoot tips was infected with TGMV. The results of meristem culture tests done by Lizarraga *et al*. (1980) [13] also indicate that viruses and viroids do not reach the shoot apex of the plant. Even though we can't say for sure that the lack of CoGMV in SAM is due to the virus not being able to reproduce in SAM, it makes more sense to think that plasmodesmata at some cell boundaries keep CoGMV from getting in to SAM, rather than CoGMV being able to get in there. The condition is similar to systemic gain gene silencing, in which an abnormal signal based on the sequence of nucleotides is sent from the bottom of the plant to the top. This causes gene silencing all over the top of the plant, except in the apical meristem and the mature leaves. This explanation is in line with that of Voinnet *et al*. (1998) [23]. In situ hybridization studies conducted by Di Serio *et al*. (2010) showed the involvement of RNA dependent RNA polymerase 6 (RDR6) in preventing PSTVd from entering the floral and vegetative meristems of Nicotiana benthamiana. This demonstrates that genetic data supports the existence of an antiviral RNA silencing pathway. In accordance with the findings of these authors, the signal does not travel into the shoot apex. There needs to be further investigation into whether the translocation of CoGMV and gene silencing information is controlled by the same set of underlying mechanisms.

We used in situ hybridization to evaluate the spread of

CoGMV throughout the plant system. All of our research findings clearly showed that CoGMV was present in the phloem during the long-distance movement (Fig. 2D). Consider the results of Arce *et al*. (1997) [1], who found that the *tobacco mosaic virus*, which makes a protein to move from cell to cell, could also need replication and phloem cells to keep moving long distances when it infects grafted tobacco plants systemically. Based on the examination of the virus's leaf localization, it was discovered that the virus was present in the leaves of infected jute plants, as seen in Figs. 2A and B. The viral genetic background had little effect on the effectiveness of mesophyll invasion in jute leaves that were systemically infected. Moreover, Sunter and Bisaro (1992) [21] asserted that effective cell-to-cell migration leads immediately to mesophyll penetration from systemically diseased plants' vascular system. The cis- and trans-acting factors (BRi and AL2) from TGMV that were required for the penetration of mesophyll were both needed for the BR1 movement protein gene to be expressed. According to Gillette *et al*. (1998) [10], the AL2 protein from *the bean golden mosaic virus*, as well as the BR1 and BL1 proteins, are less effective in *N. benthamiana* than their *tomato golden mosaic virus* counterparts. Our findings are also in line with these findings.

Hybridization signals from infected parts of floral organs were looked for to learn more about how CoGMV spreads through the parts of the plant. In situ hybridization and PCR were used to detect CoGMV in all floral sections (Fig. 3A and B). This contradicts the findings of Zhu *et al*. (2001) [26], who found the viroid in the sepals of infected plant flowers, but not in the petals, stamens, or ovaries using histochemical analysis and in situ hybridization. On the other hand, Singh (2006) [19] showed that PSTVd was found in the sepals, petals, stamens, and pistils of the flower of the tomato plant by RT-PCR.

According to the results of the CoGMV seed localization study, the virus was found in the testa tissues, the endospermic cytoplasm surrounding the embryo sac boundary wall, cotyledonary tissues, and the embryo, as shown in Fig. 4A-B. CoGMV enters the ovule wall through the main vascular strand after fertilization. The introduction of the virus in the tissues near to the micropyle allows access to the interface between the testa and the suspensor cells at later developmental stages. This explanation is in line with the opinion of Wang and Maule (1994) [25] that while the suspensor is functioning, it may operate as a route for viral transmission to the embryo proper.

5. Author's contributions

All of the experiments were designed and performed by Md Moniruzzaman Hasan, as was the preparation of the manuscript.

6. Funding information

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7. Conflict of interest

The authors declare that they have no conflict of interest regarding this manuscript and research.

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