ACCEPTANCE NOTICE OF POSTER PRESENTATION

Dear Prof. Joseph George,

On behalf of the Scientific Committee of the 32nd Conference of the Asian Pacific Association for the Study of the Liver (APASL 2023) , we are very pleased to inform you that your abstract entitled "Menadione Inhibits Activation of Primary Hepatic Stellate Cells In Vitro and Prevents Upregulation of Osteopontin and Collagen Expression

" has been accepted as the **Poster Presentation** at APASL 2023. All the information you submitted will be published on APASL 2023 congress materials.

For the advanced preparation, please **complete the reply form** below ASAP **.** The **Presenting Author** will be requested to **register online and complete the payment no later than 23:59:59 (GMT+8), 2 January 2023** or the abstract will be withdrawn.

(**Register Online:** APASL 2023)

POSTER PRESENTATION REPLY FORM

Reference No.	PN-012
Abstract Title	Menadione Inhibits Activation of Primary Hepatic Stellate Cells In Vitro and Prevents Upregulation of Osteopontin and Collagen Expression
Presenting Author	**First Name:** Joseph
	Last Name: George
Acceptance Reply	❑ Yes! I agree to present at APASL 2023. ❑ In-person ❑Virtual (for international presenter only) ❑ Sorry! I am unable to present at APASL 2023.

- Please reply to confirm your participation and contact the Secretariat promptly for amendment if above information is incorrect by **23:59:59 (GMT+8), 23 December 2023** ; after the deadline, all the content will be finalized and prepared to be published in the conference materials.
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Menadione Inhibits Activation of Primary Hepatic Stellate Cells In Vitro and Prevents Upregulation of Osteopontin and Collagen Expression

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Background: Activation and transformation of quiescent hepatic stellate cells into myofibroblast like cells with the upregulation of osteopontin (OPN) and numerous other connective tissue components marks the first step in hepatic fibrosis. Menadione, also known as Vitamin K3, generates reactive oxygen species (ROS) and produce oxidative stress leading to apoptosis in several types of tumor cells. Here, we evaluated whether low doses of menadione could inhibit activation hepatic stellate cells and prevent upregulation of OPN and collagen synthesis in the in-vitro culture of primary hepatic stellate cells.

Methods: Hepatic stellate cells were isolated from adult albino rat liver, purified, characterized, and cultured in DMEM/F12 medium with 10% fetal calf serum. The second passage of cells was cultured in 6-well plates and at 24 h the media were replaced with serum free DMEM/F12 medium containing 10 µM menadione (Sigma-Aldrich) (final concentration). The cultures were examined, phase contrast images were captured, and the cell lysate was collected at 2 h, 6 h, and 24 h after the treatment. Protein, malondialdehyde, and glutathione levels were determined in the cell lysate. Western blotting was performed for OPN and collagens type I and type III. Total RNA was extracted and the expression of OPN, a-SMA, and collagens were measured using qRT-PCR.

Results: There was no significant change in any molecule studied at 2 h after the treatment with menadione. At 6 h, there was increase in malondialdehyde and decrease in glutathione levels in the cell lysate compared to non-treated controls. The other molecules studied were not altered. The cultured cells did not show any morphological changes. At 24 h of menadione treatment, about 20% cells died due to apoptosis. There was marked decrease of glutathione and increase of malondialdehyde levels. Western blotting and qRT-PCR demonstrated that OPN, a-SMA, and collagens type I and type III expression were significantly less compared to non-treated control at 24 h.

Conclusions: The results of the present study indicate that menadione may be used as a therapeutic agent to prevent activation of hepatic stellate cells and inhibit hepatic fibrosis. However, additional in-vivo studies are required to obtain further information regarding the use of menadione to arrest hepatic fibrosis.