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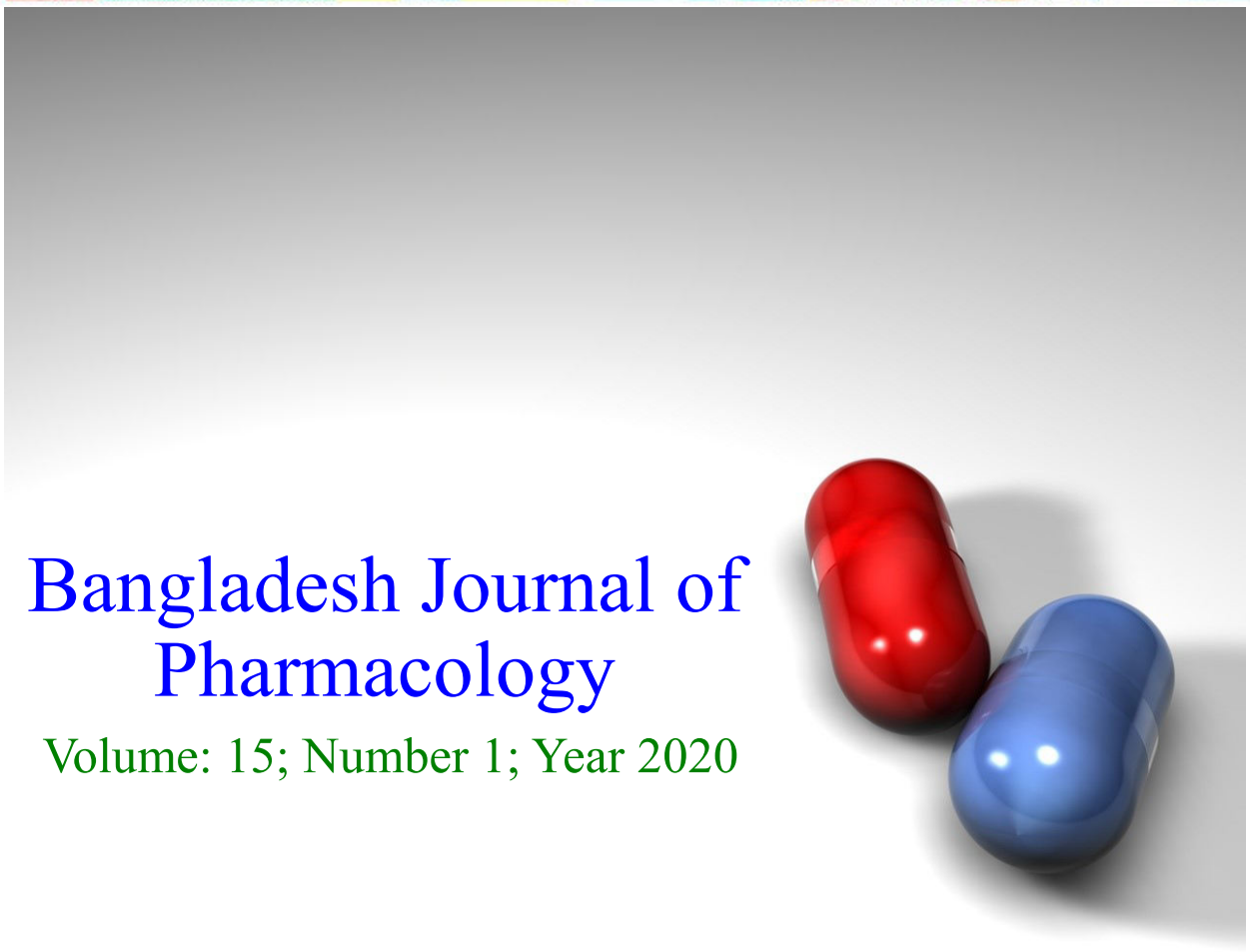

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Letter to the Editor

Effect of *Garcinia indica* against brain ischemic reperfusion in rat model

Sir,

The term, ischemia, refers to a condition which is characterized by enormous reduction of glucose and oxygen. In cerebral ischemia, the cerebral blood flow become insufficient in order to pursue cerebral metabolic activities (Ahmed at al., 2018). Both, the inflammation and oxidative stress have a promising role in cerebral infarction associated with ischemic reperfusion insult. The reperfusion insult results in an initiation of a number of events including stress oxidative, devastation of blood brain barrier, leukocytes intrusion, inflammation, apoptosis and nitric oxide discharge, etc. In this context, potential antioxidant and anti-inflammatory intermediaries might play their role to lessen the effects produced as a result of ischemic reperfusion insult. Due to lack of active treatment of ischemic reperfusion associated cerebral ischemia, researchers interest in developing some effective therapy from medicinal plants is obvious (Kalogeris et al., 2012). In order to get to know about the mechanism of cell demise and neuroprotection, a classical rat's model is widely used. In this model, the forebrain slices of the animal are bare to oxygen and glucose deficiency and reperfusion (Ca´rdenas et al., 2000). The advantages of this model are time tested as cell composition is preserved.

In comparison with the synthetic drugs, the toxicity of the naturally occurring phytochemicals is less. However, the mechanism of action, the percent of reproducible results and their accurate medicinal effects remain a question mark because the traditional herbal drugs are extracted from the crude source (Kim and Lee, 2010). For the reason aforesaid, the researchers are focusing on the active products of the herbs rather than the herb itself. It is evident that neurotrophins are imperative for subsistence, conservation and resurgence of neuronal populace in the brain. These neurotrophins are the accurate targets for plants that possess neuroprotective effects against neurodegenerative diseases (Woo et al., 2014).

Garcinia indica belongs to the family Guttiferae. It is an underexploited tree and is known as kokam, wild mangosteen, kokum butter tree, etc. Studies have shown that garcinol, an active component of *G. indica* caused the prevention of nitric oxide deposition in lipopolysaccharide-treated astrocytes. The reason was supposed to be the decrease in the expression of lipopolysaccharide-induced inflammatory intermediaries, COX-2 and iNOS (Liao et al., 2005). Reports have shown that garcinol possesses cholinesterase inhibitory potential too and the detected IC_{50} value of 0.66 µM was equivalent to that of the standard galanthamine (0.50 µM) (Lenta et al., 2007).

The *G. indica* was obtained from District Bannu and its pulverized form (100 g) was macerated in 1 L ethanol (70%). After 7 days, the filtrate was collected and concentrated using a rotary evaporator (Heidolph, Hei-VAP Core, Germany). The concentrated product was subjected to freeze drying (42.6% yield).

The animals were obtained from the National Institute of Health, Islamabad. At the day of experiment, the decapitation of the rats was done without giving anesthesia, and hippocampus were immediately isolated form the brain and kept in artificial CSF that contained (in mM): 20 NaCl, 0.5 KCl, 35 $NaHCO_3$, 1.5 $CaCl_2$, 1.3 $MgCl_2$, 1.25 Na_2HPO_4, 10 D-glucose (pH 7.4). Slices (400 µm) were cut using a tissue chopper Mcllwain Tissue Chopper TC752, Campden Instruments Ltd.). The control and experimental slices were pretreated for 30 min in the absence or presence of different concentration of *G. indica*. The experimental slices were placed in a container receiving 95% argon and 5% CO_2 whereas the control slices were treated in the presence of 95% O_2 and 5% CO_2. The duration of treatment was kept for 2 hours. Following 2 hours, the medium from both groups was changed with an artificial cerebrospinal fluid with glucose and reperfused for 1 hour. At the end of reoxygenation, MTT assay was done to assess cellular viability (Mosmann, 1983; Bahuguna et al., 2017) whereas spectrofluorophotometer (LS45, Perkin Elmar) was used to measure reactive oxygen species (Wagner et al., 2010).

The results of the current study revealed significant changes both in reactive oxygen species (ROS) quantity and cellular viability. *G. indica* presented protective effects on cellular demise at a concentration of 60 µg/mL as compared to the control slices ($p<0.001$; Figure 1A).

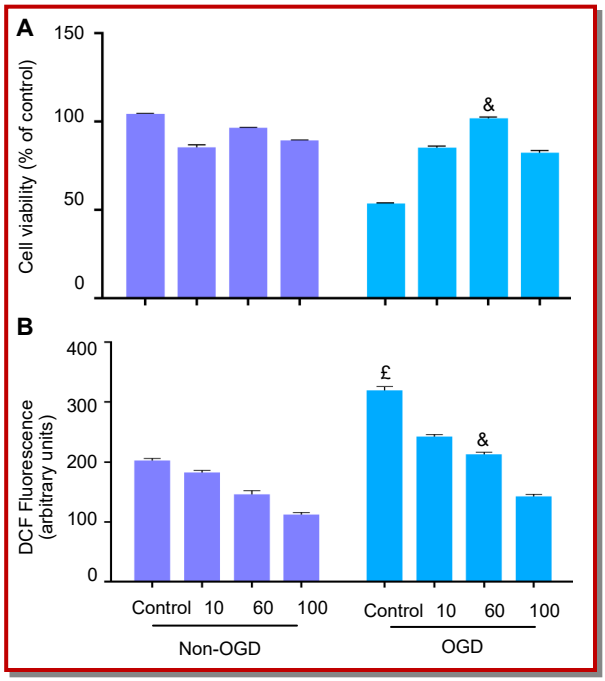

Figure 1: Effect of different concentration of *G. indica* on cellular viability (A) and oxygen and glucose deprivation-induced ROS generation (B). Data are mean ± SEM; n=4; (£) represent significant difference from untreated slices (control) at $p<0.00$ and (&) represent significant difference from slices subjected to OGD at $p<0.01$ respectively using Two-way ANOVA

Oxygen and glucose deprivation result in an increase in dichlorofluorescein fluorescence in comparison to the fluorescence noticed in the medium in control slices ($p<0.001$). Our results showed a remarkable decrease in the dichlorofluorescein fluorescence (in presence of *G. indica* extract) in a dose-dependent manner (Figure 1B).

The findings of our study suggest that *G. indica* can be a suitable candidate for the prevention and rectification of neurodegenerative disorders.

Wasim Ahmad

Department of Biotechnology, University of Science and Technology, Bannu 28100, Pakistan.

Corresponding author:

email: vazim4847@gmail.com

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