Supporting Information

Potential Therapeutic Application of Zinc Oxide Nanoflowers in the Cerebral Ischemia Rat Model Through Neuritogenic and Neuroprotective Properties

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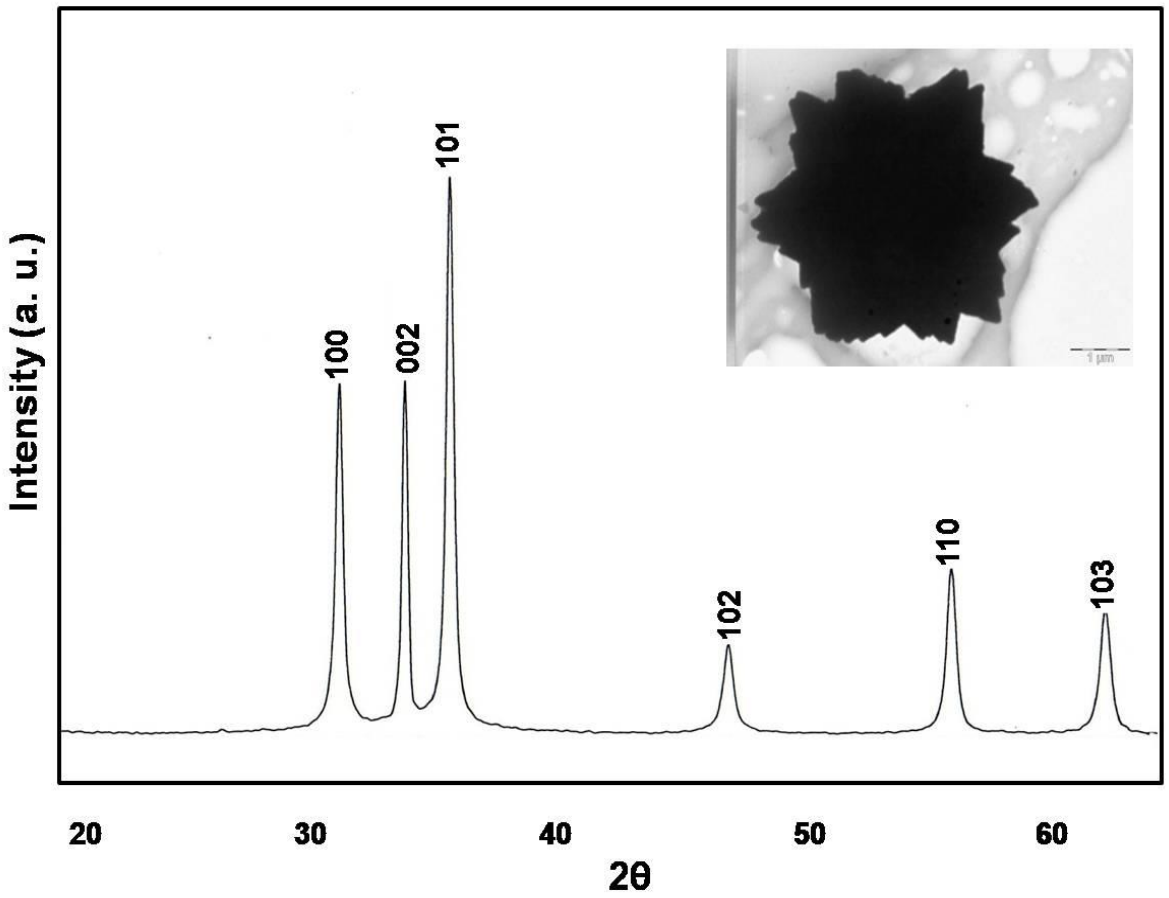

Figure S1. Characterizations of ZONF employing XRD and TEM analysis. XRD pattern demonstrates the crystalline structure of ZONF having wurtzite phase. The inset TEM image shows the flower shaped nanoparticles.

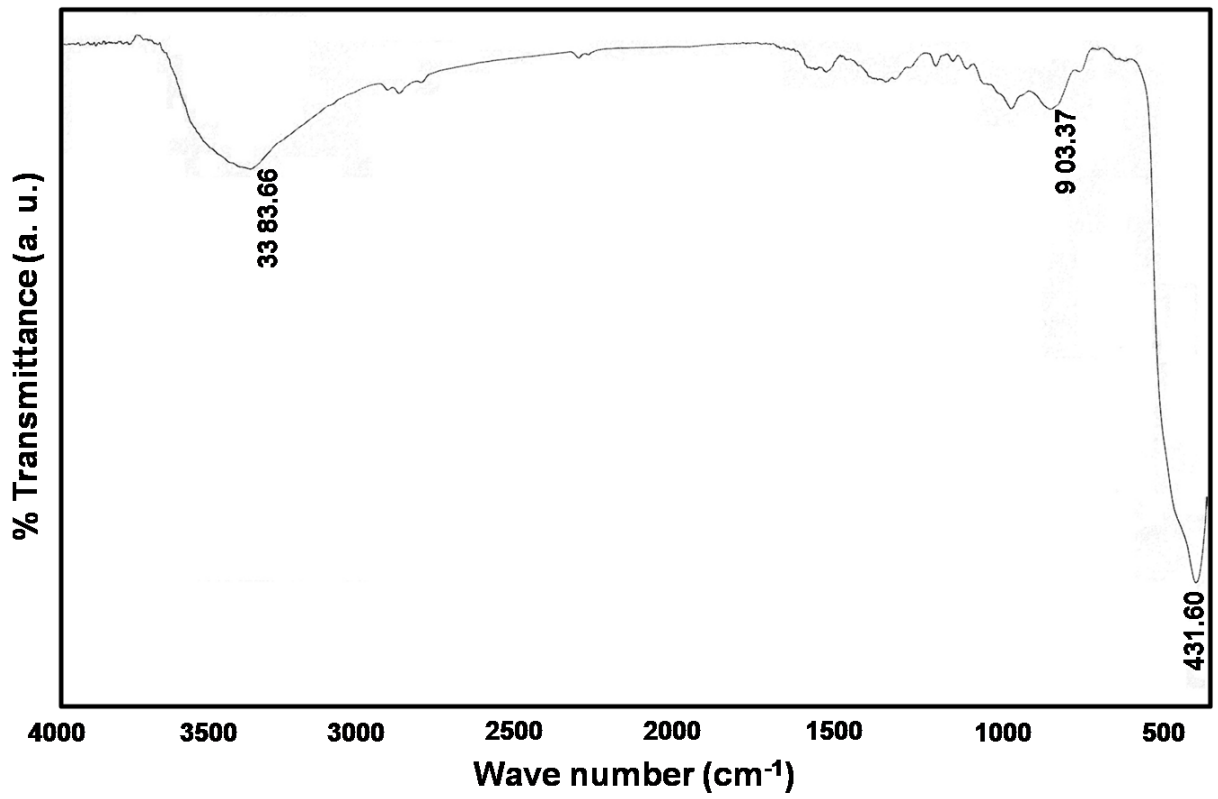

Figure S2. FTIR analysis of as synthesized ZONF. The characteristic peaks appeared at 3383 cm^{-1} corresponds to O-H stretching, while peaks at 903 cm^{-1} and 431 cm^{-1} can be referred to the typical Zn-O stretching.

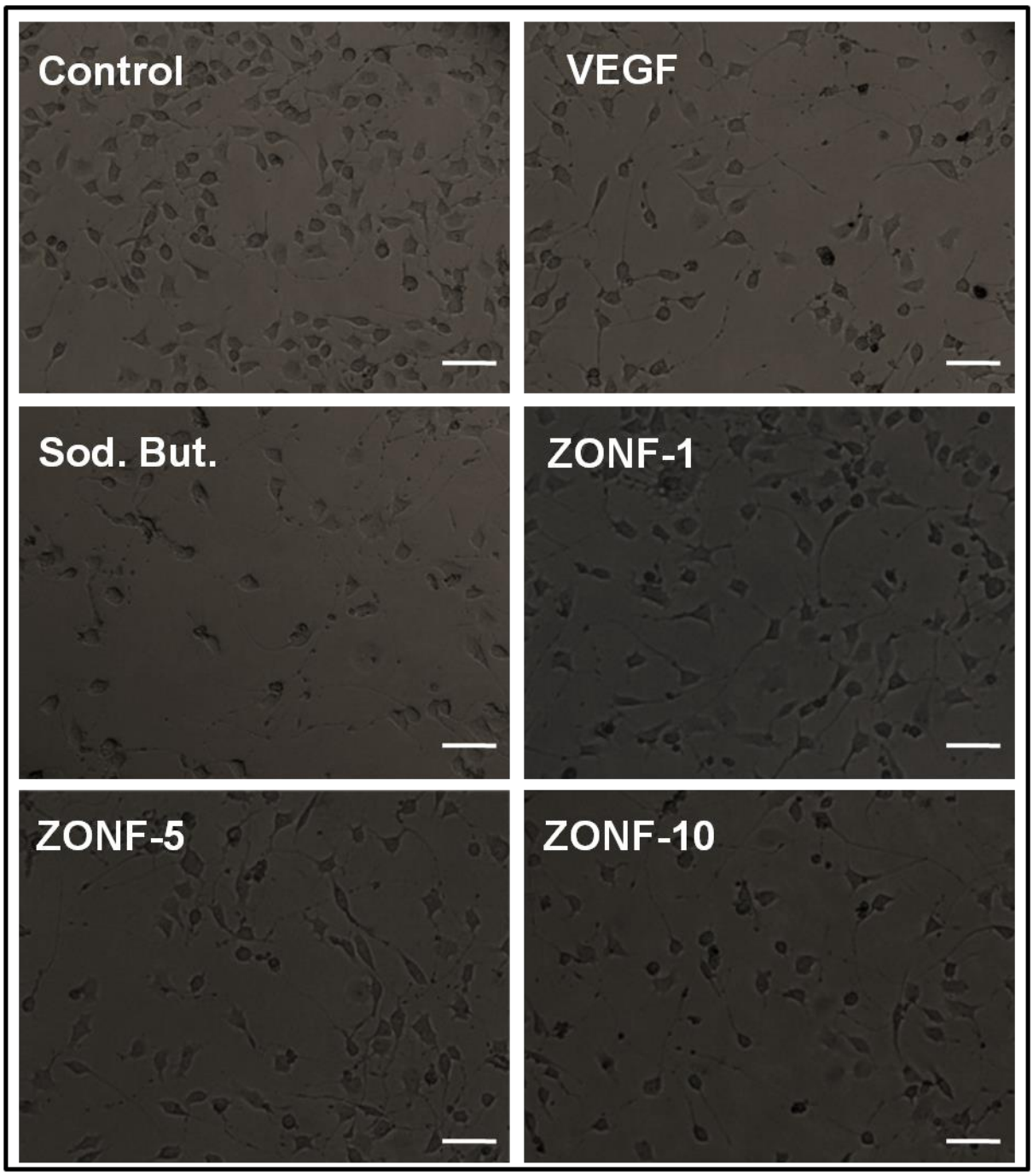

Figure S3. Representative images of Neuro 2a cells, treated with ZONF (1-10 µg/mL) for 48 h, clearly exhibit the neurite outgrowth as compared to control experiment. Scale bar = 50 µm.

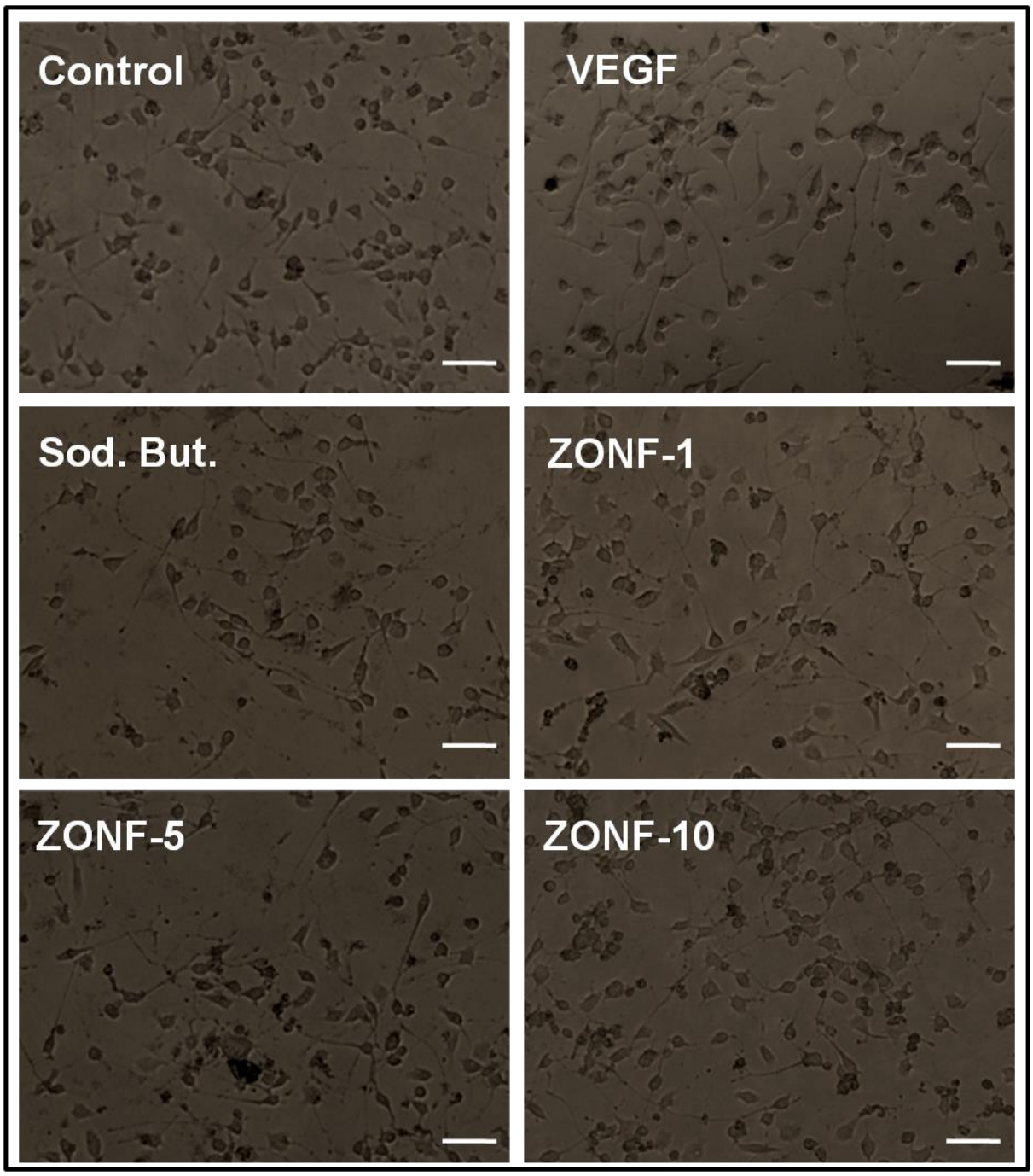

Figure S4. Representative images of Neuro 2a cells, treated with ZONF (1-10 µg/mL) for 72 h, clearly exhibit the neurite outgrowth as compared to control experiment. Scale bar = 50 µm.

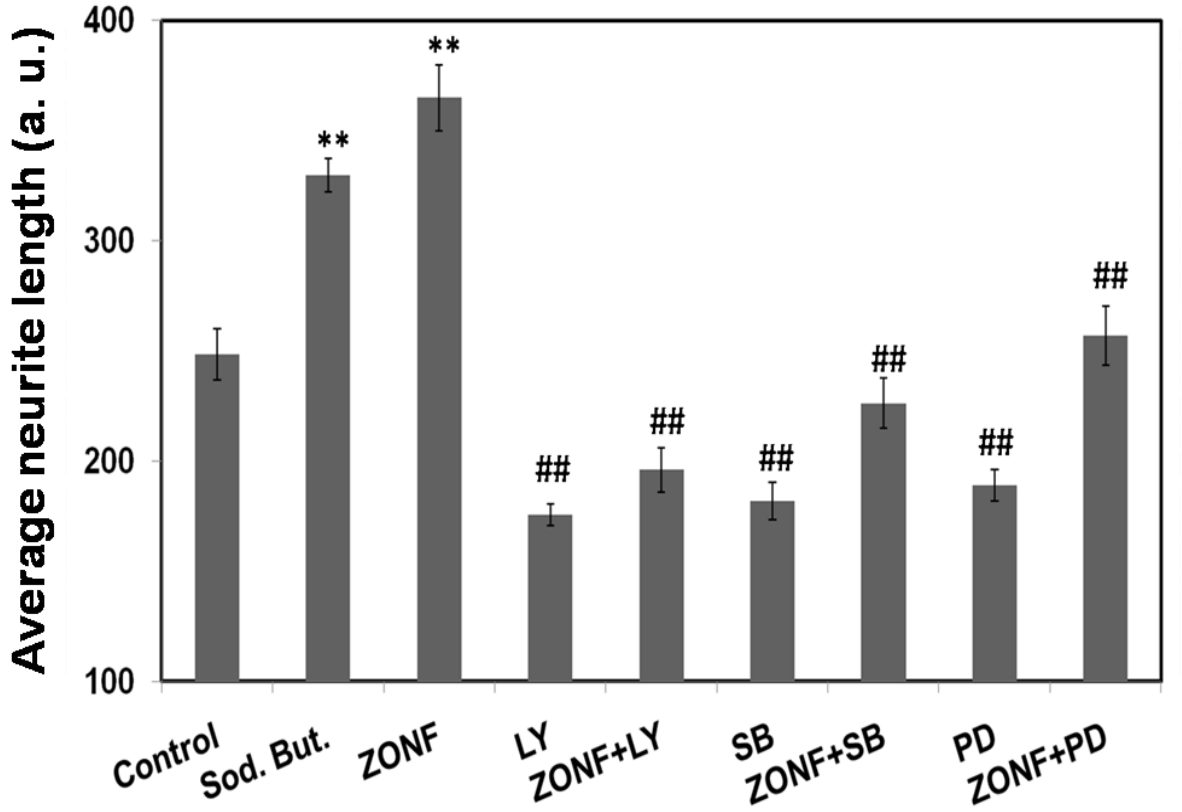

Figure S5. Mechanistic study in Neuro 2a cells reveals that LY294002 (LY: 20 µM, 48 h), SB202190 (SB: 20 µM, 48 h) and PD98059 (PD: 40 µM, 48 h) significantly attenuated the ZONF (10 µg/mL, 48 h) induced neurite outgrowth, suggesting the role of PI3K/Akt and p38MAPK/ERK1/2 signaling pathways behind nanoflowers mediated neuritogenesis. Values are mean ± SEM of three independent experiments; *p < 0.05, **p < 0.01 as compared to control and #p <0.05, ##p <0.01 as compared to ZONF.

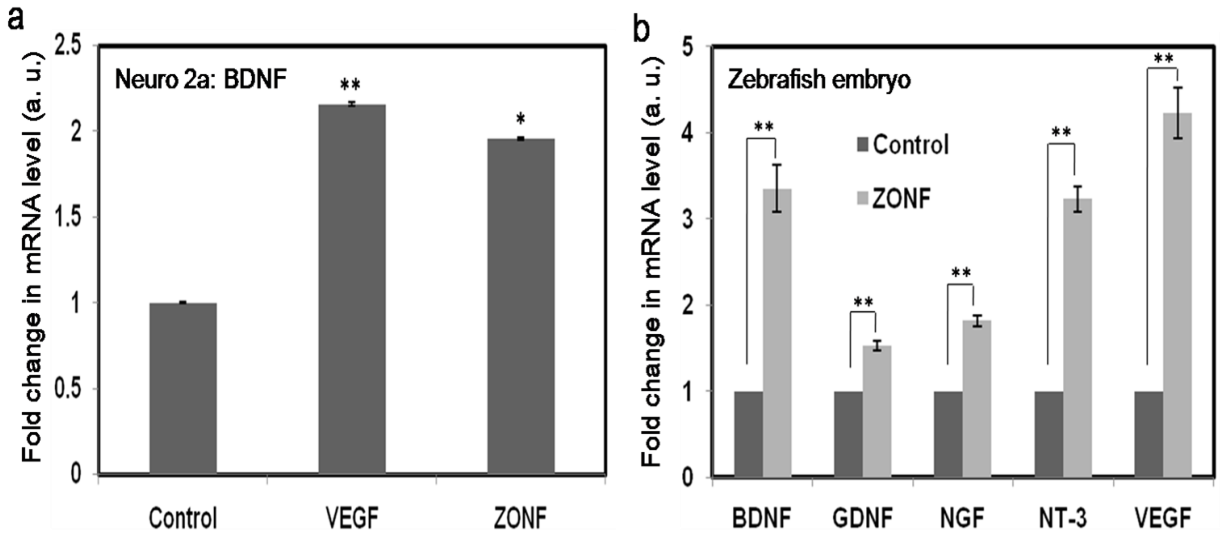

Figure S6. *In vitro* (Neuro 2a cells) and *in vivo* (Zebrafish embryos) real time PCR studies. **(a)** This study shows the elevation of cellular mRNA expression level of BDNF in Neuro 2a cells in response to ZONF (10 µg/mL, 48 h) as compared to control experiment, suggesting the role of BDNF during nanoflowers driven neuritogenesis. Values are mean ± SEM of three independent experiments; *$p < 0.05$, **$p < 0.01$ as compared to control. **(b)** The result exhibits the upregulation of mRNA expression level of neurotrophic factors BDNF, GDNF, NGF, NT-3 and VEGF in Zebrafish embryos in response to ZONF (5 µg/mL, 24 h), suggesting the neuritogenic potential of nanoflowers for *in vivo* system. Values are mean ± SEM of three independent experiments; *$p < 0.05$, **$p < 0.01$ as compared to control.

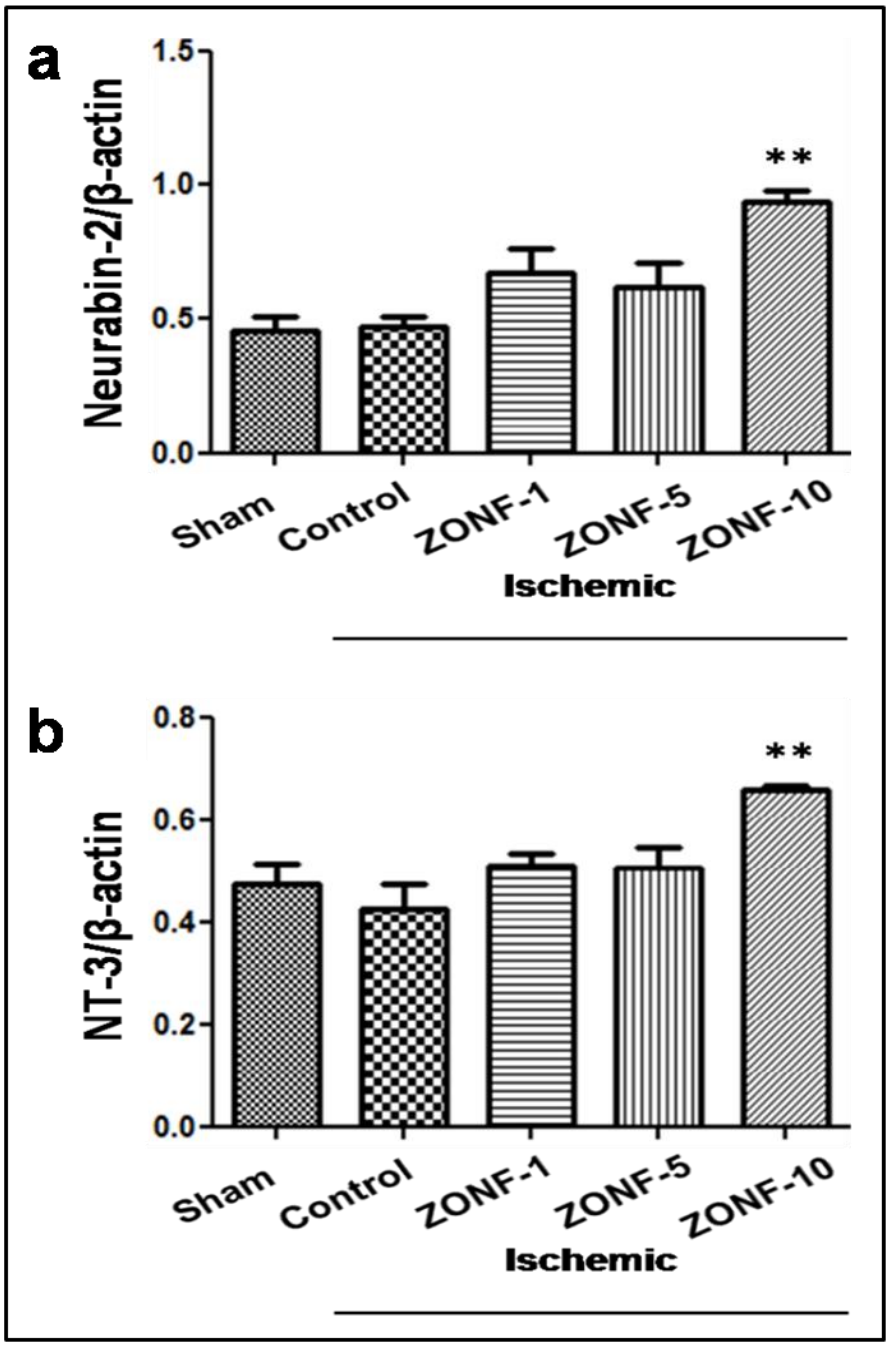

Figure S7. Quantification of western blot using ImageJ analysis software and normalization by β-actin using brain tissue of Fischer rats. Here β-actin was used as a loading control. This study exhibits that ZONF (10 mg/kg b.w.) could significantly upregulate the Neurabin-2 and NT-3 levels in ischemic rats, indicating their important role behind neuroprotective activity of ZONF. Values are mean ± SEM of three independent experiments; *p < 0.05, **p <0.01 as compared to control.

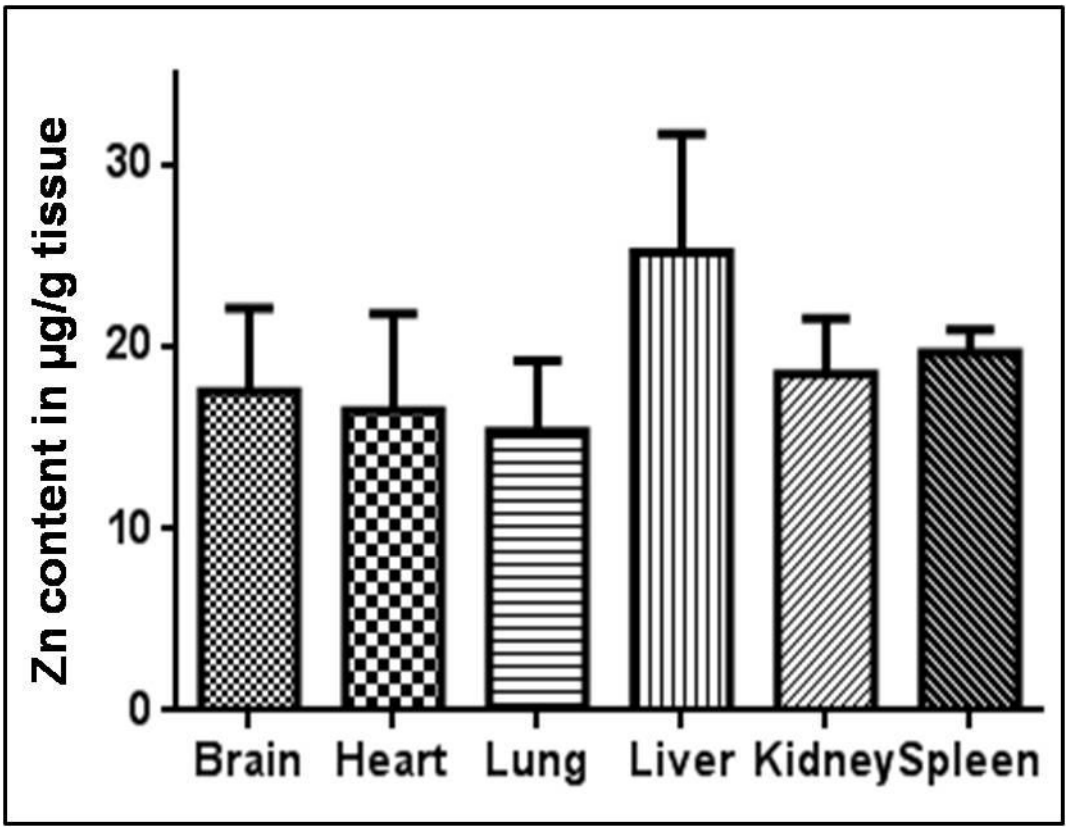

Figure S8. Organ bio-distribution of ZONF in Fischer rat using ICP-OES technique. Zinc was broadly distributed in brain, heart, lung, liver, kidney and spleen in rats administered with ZONF (10 mg/kg b.w.). The major accumulation of zinc was observed in liver that might be due to the intraperitoneal administration of ZONF, leading to more entrapment of nanoflowers in reticuloendothelial system. Values are mean ± SEM of three independent experiments.